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SOME NOTES ON PARASITIC AND OTHER DISEASES OF FISH.

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Plate II.

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In the following notes no attempt has been made to enter into the pathology or etiology of the diseases described, as only isolated cases have been available for investigation.

I am much indebted to the Council of the Marine Biological Association of the United Kingdom for their kindness in granting me a table at their Plymouth Laboratory.

A new species of Pleistophora *invading the muscles of a Cod.*

In January of this year a cod caught off the Iceland coast was sent to me for examination.

Diffuse pigmented areas of a brownish colour were present in various parts of the muscular tissue. The boundaries of these areas were ill defined, but the pigmentation was more intense in the central portions, where the muscular tissue showed marked degeneration.

Unfortunately only portions of the infected muscle were sent for examination, and none of the viscera were available. The specimen was sent unpreserved, and the tissues were somewhat degenerated when it arrived.

On teazing out portions of the pigmented areas, minute spherical vesicles, and the 'yellow bodies' so often associated with infections by Myxosporidia, were visible under the microscope. Sections showed that the muscular tissues in the centre of the lesions were much degenerated. The muscle fibrils were partially, or in places completely, replaced by a finely granular material contained within the sarcolemma: the shape of the fibrils was seldom altered, but the sarcous elements within the sheath were usually destroyed.

In the regions surrounding the areas of most acute infection some inflammation was present, and the interfibrillar spaces contained many leucocytes: the striation of the muscle was also somewhat less pronounced than in the normal tissue. The 'yellow bodies' were plentiful in the centre of the lesions.

Small spherical vesicles, averaging about 30 μ in diameter, were present in the interfibrillar tissue, or actually within the fibrils. Each vesicle was bounded by a thin structureless transparent membrane, resistant to potassium hydrate solution. Each contained a mass of protoplasm showing stages of division into a large number of spores (more than 8). These vesicles appear to resemble the condition described by Gurley as occurring in *Pleistophora typicalis* (found in the muscles of *Cottus scorpio*), in which the pansporoblast membrane is sub-persistent as a polysporophorous vesicle which represents the pansporoblast stage.

The spores were oval in shape, about 3 μ long, and 2·5 μ broad; they contained a small granular area, staining with haematoxylin and basic dyes, which probably represents the nucleus. No polar capsules could be seen, but this was possibly due to the imperfect preservation of the specimen.

As in the case of *Pleistophora typicalis,* the trophozoite stage was not observed. Some small protoplasmic masses, containing several definite nuclei, were found within the fibrils and in the interfibrillar tissue and these probably represent stages in the formation of the pansporoblast. Adopting Minchin's classification, it would appear that this parasite belongs to the

Order	*Myxosporidia* (Bütschli)
Suborder	*Cryptocystes* (Gurley)
Family	*Glugeidae* (Théloran)
Section	*Oligosporogenea* (Doflein)
Genus	*Pleistophora* (Gurley)
Species	? *Nov.*

For an account of the literature of the subject see Gurley, R. R. (1894). The Myxosporidia or Psorosperms of Fishes. Commissioners' Report of the United States' Commission of Fish and Fisheries for 1892. Part XVIII. p. 194.

On bacterial infection accompanying the invasion of the swim bladders of trout by Cystidicola farionis (G. Fischer).

Some Rainbow and Brown Trout belonging to the Hon. Sydney Holland, Royston, Herts., have for some time been dying in considerable numbers with symptoms of an infection of the swim bladder.

Shortly before death they are found swimming near the surface of the water and finally die with their heads downwards and body nearly perpendicular. The swim bladder in every case was infected with the threadworm *Cystidicola farionis.* The condition has been fully described by Mr A. E. Shipley in his Note on *Cystidicola farionis* [Fischer] (*Parasitology*, Vol. I. June 1908, pp. 190–192).

Some of these trout were preserved in formalin and sent to me for examination at the Plymouth Laboratory of the Marine Biological Association.

In every case from 10 to 30 specimens of *Cystidicola* were present in the swim bladder, and in many cases a small amount of a fibrino-purulent material was adherent to the walls. Smears of this exudation were made, fixed by heat, and stained with methylene blue. Under the microscope these slides showed many leucocytes, fibrin, and large numbers of bacteria. The leucocytes were somewhat degenerated but many of them showed phagocytosis: many different forms of bacteria were present. Preparations of the walls of the swim bladder showed dilatation of the capillaries with transmigration of leucocytes and some fibrin formation. The condition was typically that which would be produced by infection of a serous cavity with bacteria.

Through the kindness of Mr Holland I was able to go to Royston and investigate the matter on the spot. Eight fish were caught and dissected, of these seven were infected with *Cystidicola.* These seven all showed signs of inflammation of the swim bladder and smears showed the presence of many bacteria and distinct pus formation: the remaining fish, a Brown Trout, was free from *Cystidicola* and the swim bladder showed no signs of inflammation nor could any bacteria be detected.

In each case before opening the swim bladder, the wall was well

seared with a red hot seeker, a sterile platinum needle introduced, and sterile sloped tubes of peptonised fish gelatine were inoculated. The tubes inoculated from the seven infected fish showed a free growth of bacterial colonies in the course of a few days, whilst the one inoculated from the remaining fish, which was free from *Cystidicola*, remained sterile. At least twelve different species of bacteria were present, distinguishable by their shape, form and colour of the colonies, powers of liquefying gelatine, staining reactions, etc.

The gas from the swim bladders of two of the infected trout was collected and an analysis gave

Carbon dioxide (absorbed by potash)	1·5 %
Oxygen (absorbed by potassium pyrogallate)	0·0 %
Nitrogen (by difference)	98·5 %

These fish had been caught and allowed to die in the air, so it is possible that any oxygen present during life may have been used up during the process of asphyxiation. Mr F. G. Richmond, of the Surrey Trout Farm and United Fisheries Co. Ltd., kindly sent me some healthy fish, uninfected with *Cystidicola*, for purposes of comparison: cultures from the swim bladders of these fish in every case remained sterile. It would thus seem probable that bacterial infection may be conveyed by *Cystidicolae* in their migration into the swim bladder of the trout, and it is possible that death may be caused by the introduction of pathogenic organisms in this manner. It is easily conceivable that any agent causing acute inflammation of the swim bladder, with the consequent dilatation of its blood vessels, would produce excessive liberation of dissolved gases from the blood, and thus by undue distention of the swim bladder disturb the equilibrium of the fish.

With regard to the mode of entry of the parasite, it is probable as Shipley (*loc. cit.*) suggests, that they enter along the ductus pneumaticus, but it is also possible that in some cases they migrate directly from the intestine. I recently examined a roach containing *Cystidicolae* both in the intestine and swim bladder, in which the parasite could be seen in various stages of development encysted in the intestinal wall, mesentery, and subperitoneal tissue, and so apparently making its way from intestine to swim bladder directly through the tissues.

Primary Carcinoma in the Liver of Rainbow Trout.

Plate II. Fig. 1.

Two out of the eight Rainbow Trout that were examined in connection with the disease of the swim bladder previously described, were found to have tumours in the liver.

In one case the tumour was about the size of a large pigeon's egg, and was situated in the posterior part of the left lobe of the liver. It was of a yellowish white colour, and the surface appeared somewhat nodulated. On cutting it open two portions could be distinguished, a hard white fibrous looking area, and a softer part which showed small red points on the cut surface. The growth was not capsulated. The fish was a female of about $1\frac{3}{4}$ lbs. but was considerably wasted and in bad condition.

In the other case the tumour was about the size of a pea, situated in much the same position as the former growth, unencapsuled, and homogeneous in structure. The fish was a male of about 1 lb. and appeared in good condition.

The tumours were fixed in 5 % formalin, and serial sections were cut. Some were stained with haematoxylin and van Gieson's stain, others with methyl violet, safranin, etc.

On examining the sections it was seen that both the growths were very similar in structure: they only differed slightly in vascularity and in the amount of fibrous tissue present. Fig. 1 shows a section of the smaller growth. Toward the upper part of the figure the normal liver cells are seen, below this the cells and their nuclei are of a markedly larger size, and the nuclei show a great number of mitoses. The abnormal cells had slightly different staining reactions to the normal tissue, but they showed none of the staining peculiarities of amyloid degeneration. Scarcely any interstitial tissue was present in this tumour. Sections of the larger tumour showed an almost identical structure, with the exception that the harder portion contained more interstitial material, and the softer part was more vascular.

No secondary metastases were found.

Dr Bashford of the Imperial Cancer Research Fund, and Mr Strangeways of the Pathological Department, Cambridge, have both been kind enough to examine sections of these growths and are agreed that the condition is one of primary adeno-carcinoma of the liver.

Dr. Bashford states that it is the first case of the kind he has seen in fish.

A superficial New Growth occurring in a Dog-Fish.

Plate II. Fig. 2.

Along the right side of a male speeimen of *Scyllium canicula* there was a diffuse growth of greyish colour, stretching from about the level of the pelvic fins nearly to the tail. The growth consisted of multiple nodules which had coalesced over a great part of the affected area: in many plaees it had degenerated leaving the sub-dermal tissues exposed. The seales with their basal plates had disappeared in every part of the area affeeted by the growth.

The right side of the fish was normal.

The distribution of the growth roughly eoincided with that of the mucous eanals of the lateral line, but it had spread in all directions over an area not normally supplied with these eanals.

Sections (Fig. 2) showed a condition in many ways resembling an adeno-carcinoma. The growth consisted of irregular tubules eontaining masses of small cells. The walls of the tubules were eomposed of fibrous tissue, and the tubules themselves were nearly, or in some eases quite, filled with small oval eells. These cells possessed a relatively large amount of cell body and many of their nuelei showed various stages of mitosis. The eells near the walls of the tubules were more elongated in shape than those near the lumen of the tube.

The dermal tissue underneath the affeeted area showed a eertain amount of inflammation.

Unfortunately only the posterior part of the body of the fish was available for examination: the head and viseera had been destroyed before the specimen eame into my hands so it was not possible to determine whether secondary metastases had been formed.

Dr Lazarus Barlow, of the Middlesex Hospital Cancer Researeh Laboratories, very kindly examined sections of the growth for me. He is of the opinion that though in many ways the tumour resembles an adeno-carcinoma, yet from a consideration of the histologieal details of the abnormal cells and their arrangement, it is probably of an endotheliomatous nature and may be non-malignant.

On a superficial examination of the seetions the resemblanee to an adeno-eareinona is very marked. The structure of the mueous canals of the lateral line appears to be reprodueed with an abnormal proliferation of the columnar epithelial cells lining the glands. New mueous eanals appear to have been formed and to ramify in all directions beyond the

area normally supplied by the canals of the lateral line. Many of these new formed glands do not open superficially but are covered by the epithelium of the skin. The basal cells near the walls of the tubules also appear to resemble somewhat the type of columnar epithelium found in the mucous canals. On the other hand, Dr Lazarus Barlow points out that the abnormal cells contain a relatively large proportion of protoplasm for the size of their nuclei, and that the nuclear chromatin has a tendency to radiate from a central condensation to the periphery of the nucleus. The cells thus approximate nearer to an endotheliomatous type than an epitheliomatous. Also in these sections the abnormal cells in the cell masses seem to have a tendency to arrange themselves round a central lumen as though trying to reproduce the structure of a capillary or lymphatic. This condition is just noticeable but not well defined.

A Myxo-fibroma originating in the Jaw of a Trout.

Plate II. Fig. 3.

A slow growing tumour was observed protruding from the lower jaw of a trout in the Duke of Bedford's fish hatchery at Endsleigh. This slowly increased until it was of about the size of a large hen's egg. Finally it sloughed off, taking with it a portion of the jaw.

The tumour was white and fibrous in appearance.

Sections (Fig. 3) showed that the growth consisted of strands of white fibrous tissue between which were spaces traversed by delicate fibrils and containing stellate cells with oval nuclei. The section presented the typical appearance of a myxo-fibroma.

The fish completely recovered after the sloughing of the growth.

Pericarditis in a Turbot.

A post mortem examination was made of a turbot which died in the Aquarium of the Marine Biological Association at Plymouth. The fish was probably about 15 years old. It was found that the peritoneal cavity was distended with fluid, the liver showed marked venous engorgement, the veins were everywhere distended, and the tissues were flabby and distended with fluid. The condition was typically that which would be produced by cardiac dropsy.

The pericardial sac was enlarged to about eight times the size of that of a similar fish of approximately the same size and age. On opening the

pericardium, the cavity was seen to be filled with a dense mass of fibrin and a slightly cloudy liquid. The heart was much hypertrophied, but no endocarditis or valvular disease was present. The walls of the heart, with the exception of the region of the apex of the ventricle, were tightly bound down to the walls of the pericardium by the fibrin formation. The hypertrophy of the heart and partial organisation of the fibrin showed that the condition was of long standing.

Before the pericardium was opened, the wall was seared with a red hot seeker, a platinum needle introduced, and cultures made on sloped peptonised fish gelatine.

In the course of a few days a number of cream coloured colonies developed: these did not liquefy the gelatine. Subcultures in broth, in the course of 48 hours at the room temperature, became cloudy and formed a yellowish sediment at the bottom of the tube.

These growths consisted of pure cultures of a slender diplobacillus with rounded ends. The bacilli were about $1{\cdot}75\,\mu$ in length and arranged end to end: they stained with the ordinary basic dyes but not by Gram's stain. Sections of the fibrinous mass in the pericardium showed fibrin, which was partially organised in the region nearest the pericardial wall, leucocytes, and the diplobacilli described above.

A Recurrence of the 'Salmon Disease' in the Colne.

In May of this year several specimens of chubb and roach which had died infected with a white fungus growth were sent to me for investigation.

It was reported that large numbers of trout, chubb, roach, and eels were dying in the Hertfordshire Colne from the same cause.

Before death the fish swam slowly near the surface of the water and seemed very weak, their sense of equilibrium appeared to be diminished or lost, and the gill covers moved rapidly as though the fish were being asphyxiated. Small patches of white fungus usually appeared first on the head, adipose fin, and bases of the other fins: these then spread along the sides and coalesced until finally nearly the whole fish was covered with the growth. The epidermis and scales were more or less completely destroyed in the affected areas.

The specimens were sent packed in ice.

Examination of the fungus showed that it resembled *Saprolegnia ferax*: cultures from it were made in flasks containing boiled sterile

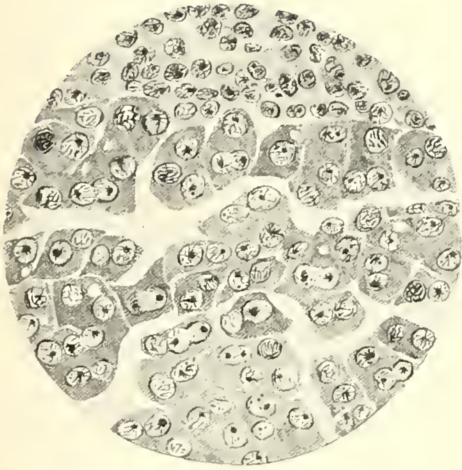

Fig. 1.

Fig. 2.

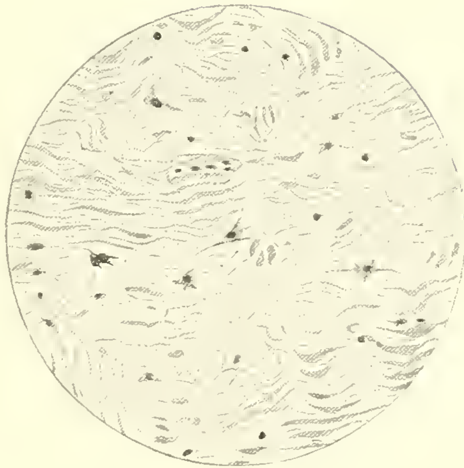

Fig. 3.

Fig. 1. Primary adeno-carcinoma of liver of trout. ×300.

Fig. 2. Superficial growth on a dog-fish. Probably of an endotheliomatous nature. ×80.

Fig. 3. Myxo-fibroma growing from jaw of a trout. ×250.

sheep's liver. A plentiful growth resulted which soon developed the typical sporangia of *Saprolegnia ferax.*

Cultures on peptonised fish gelatine were made from the superficial and subcutaneous tissues of the fish and resulted in a rapid growth of bacterial colonies, some of which liquefied the gelatine.

These colonies developed at first as small grey dots surrounded by a partially liquefied area: in later stages all the gelatine became liquefied and the individual colonies were indistinguishable. The colonies were composed of actively motile, short, thick bacilli with rounded ends, usually arranged in pairs, end to end. They stained readily with the usual stains, but did not retain Gram's stain. Subcultures grew profusely in from 18 to 24 hours at the room temperature (about 16° C.), and caused rapid liquefaction of the gelatine: they also grew profusely but less rapidly at 0° C.

Peptonised fish broth, inoculated from these cultures, showed cloudiness throughout in from 18 to 24 hours, and developed a delicate pellicle on the surface which adhered to the sides of the tube.

Sections of the subcutaneous tissues, cut perpendicular to the surface and stained with Löffler's methylene blue, showed that the tissues under the affected areas were crowded with the diplobacilli described above: in the deeper portions these diplobacilli were present alone, but in the more superficial parts there was a mixed infection. A few weeks after receiving these fish I was given the opportunity of visiting the Colne and of investigating the matter on the spot. I repeated the experiments with cultures taken from fish in the earlier stages of the disease and again isolated the same bacillus.

There seems little doubt that the diplobacillus was the *Bacillus salmonis pestis* described by J. Hume Patterson in the Parliamentary Report on the Salmon Disease, presented by the Fishery Board for Scotland (1903), and that the fungus *Saprolegnia ferax* had attacked the necrosed tissue produced by the bacterial invasion.

This combined infection is what is commonly known as the Salmon Disease, which produced such great mortality among salmon, trout and other fish in 1877, 1882, and following years.

HERPETOMONAS ASPONGOPI.

By W. M. ADERS, Ph.D.

(2 Figures.)

(From the Wellcome Research Laboratories, Khartoum.)

During the winter of 1908, when working in the Wellcome Laboratories at Khartoum, Dr Balfour proposed that I should examine a number of parasites both of plants and animals in order to ascertain if the former were infested with any other species of parasite; in other words, to study what has been termed hyper-parasitism.

The first parasite examined was the "Melon Bug," *Aspongopus viduatus*, a large hemipterous insect which causes considerable damage to the melon crops in Khartoum. The adult female lays her eggs on the leaves of the melon plant. The eggs are green in colour and usually number from 12 to 15. The young larvae in the winter months hatch out in about 12 days; they are bright crimson in colour, shed their skins several times, and develope into nymphs. The nymphs vary much in colour, some being of a bright red, whilst others are blackish. The bugs, if supplied regularly with fresh food, will live well in captivity.

The alimentary tract (see Fig. 1) of the adult *Aspongopus* consists of a short narrow oesophagus which opens into a large sacculated crop: this adjoins the large round stomach, directly behind which is another dilatation, the mid-gut: this is somewhat pear-shaped, and brightly coloured, and it is continuous with the small intestine which is generally much coiled. At the junction of the small intestine and the colon four long narrow malpighian tubes arise: and the colon terminates directly in the rectum. The salivary glands are easily recognised as a pair of white glistening bodies, each consisting of five acini which open into the narrow oesophagus. In adult females the ovaries which are large green grape-like bodies, situated on either side of the body cavity, are

very conspicuous. The whole of the abdominal organs are in close relation to the fat bodies and tracheal tubes. Seven out of 120 adults were found to be infected; many nymphs were examined but always with a negative result.

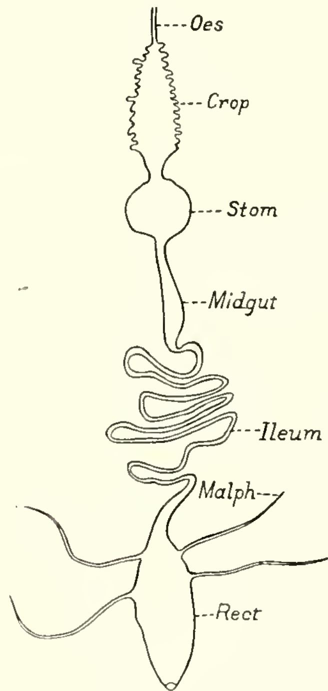

Fig. 1.

Methods.

The intestinal tract was removed and placed in a watch glass containing normal saline solution, a small piece of the crop was cut away and the contents placed on a slide and examined. In many cases swarms of herpetomonads were seen actively swimming about. Other pieces of the crop were now taken and their contents smeared out for staining. The films thus obtained were fixed in osmic acid vapour for a few seconds and then placed for ten minutes in absolute alcohol. I found Leishman's stain the best to use, as with Giemsa's it was very difficult to stain the flagella satisfactorily.

Structure and Life-Cycle of the Parasite.

I shall commence by describing what I regard as the resting stage of the parasite. Bodies probably representing this stage were often found in the crop and on two occasions in the faeces. They are oval,

round, or pear-shaped bodies (Fig. 2, *a*) measuring from 1 to 2 μ in length. Their protoplasm stains blue and in parts is very finely vacuolated. These round bodies contain two large nuclei somewhat diffuse in structure. Nearly the whole of the cell is taken up by the nuclei. A general enlargement of this resting body is the first stage towards further development. The two large nuclei divide thus forming four nuclei (Fig. 2, *b* and *c*). Immediately after this the cell divides by

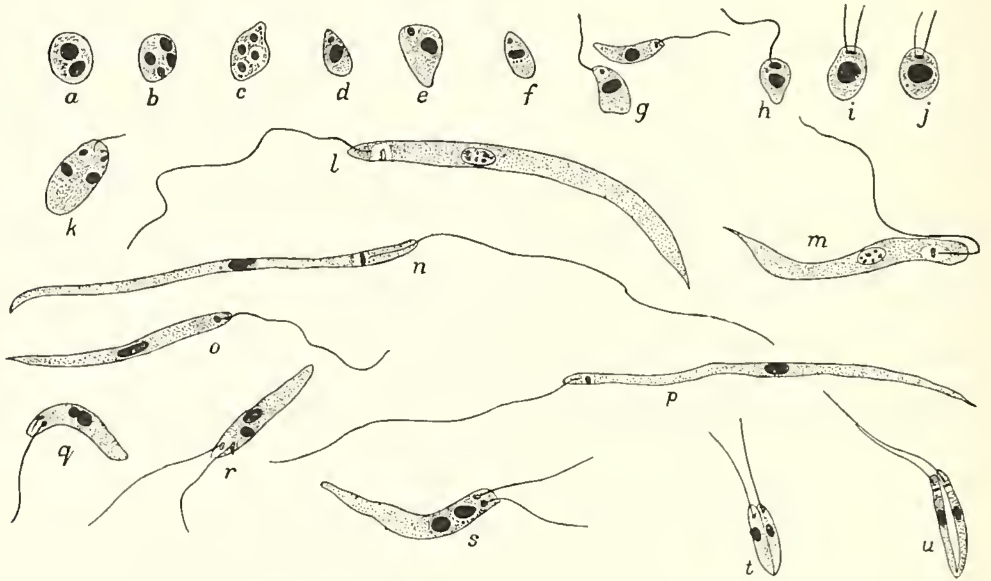

Fig. 2. All the drawings were made with a camera lucida.
Magnification: $\frac{1}{12}$th oil immersion and 4 eyepiece (Zeiss) × 940.

a	Resting stage.
b and *c*	Division of resting stage showing four nuclei.
d, *e* and *f*	Young form somewhat resembling a Leishman-Donovan body.
g and *h*	Two young forms with flagella.
i and *j*	Two young forms showing division of blepharoplast and flagella.
k	Young form with blepharoplast and macronucleus divided.
l, *m* and *n*	Adult flagellates.
o and *p*	Long thin form of adult flagellate.
q	Dividing form with macronucleus divided.
r and *s*	Dividing forms with macronucleus, blepharoplast and flagellum divided.
t and *u*	Last stages of division.

transverse fission. In this last stage one of the nuclei decreases in size and probably becomes the blepharoplast of the young immature parasite (Fig. 2, *d*, *e* and *f*). I have constantly found these bodies present in my preparations and believe that they are the resting stage of *Herpetomonas aspongopi*, but as they were only found in the faeces on two occasions it

is possible that their significance may have been mistaken. The young immature parasite is generally pear-shaped, and however formed is easily recognisable (Fig. 2, *d* and *e*). Its nucleus stains pink and is situated at the rounder end of the parasite. The protoplasm stains blue and may appear somewhat vacuolated. When highly magnified indefinite chromosomes can be made out, but it is not possible to count them. In the young parasites the blepharoplast measures about $\frac{1}{3}$ the size of the nucleus; it is almost always circular in shape and stains deep red. Owing to its great affinity for the stain no inner structure could be demonstrated. There was no other structure visible in these young forms. In the next stage the young parasite appears larger. Its form is elongated and the flagellum grows out from the micronucleus (Fig. 2, *g* and *h*). Division by longitudinal fission may now commence (Fig. 2, *i* and *j*). The parasites now rapidly become elongated until the adult stage is reached.

A typical adult parasite (Fig. 2, *l*, *m* and *n*) measures about 18 μ in length and from 2 to 3 μ in breadth at its widest part. Its posterior end is usually somewhat pointed, although I have seen a few with blunt ends. The body is cylindrical with a blunt anterior end. The protoplasm stains very evenly, there being no vacuolated areas. One unstained area is present around the blepharoplast. The nucleus is generally spherical in shape and is placed centrally, but occasionally it is situated behind. The nucleus shows great affinity for the red constituent of the Romanowsky stain, but in more or less decolourised preparations chromosomes can be distinctly made out. The blepharoplast is more rod-shaped than round: it stains very darkly and is situated at the anterior portion of the flagellate. In many parasites it has a double appearance suggesting commencing division. The flagellum measures about 13 μ in length; it consists of one stout filament which arises from an achromatic space somewhat anterior to the blepharoplast and passes out at the anterior end. The intracellular portion of the flagellum does not differ from the rest, and no basal granule could be detected. On examining a flagellate which is about to commence dividing (Fig. 2, *q*) it can be seen that the nucleus is somewhat enlarged; it does not stain so readily, and in some cases chromosomes are recognisable. The blepharoplast is thickened and somewhat elongated. It was not possible to ascertain if the second flagellum grew out of the micronucleus, or if it were formed by the splitting of the original flagellum. After close examination of many specimens it seems most probable that the new flagellum grows from the micronucleus (Fig. 2, *q*), at a later stage the

blepharoplast splits transversely (Fig. 2, *r*) and the two halves become separated. The nucleus now becomes elongated and it undergoes division (Fig. 2, *r* and *s*). A line is seen which commences at the root of the blepharoplasts and runs to the posterior end of the parasite. Along this line the parasite divides (Fig. 2, *t*), the anterior end of the parasite separating first, the cleavage later extending to the posterior end (Fig. 2, *u*). It is quite common to see all stages of division in the crop in good infections. The parasites evidently divide many times, as division was observable in quite small forms, and also in many larger forms which evidently gave rise to the long thin form (see Fig. 2, *o* and *p*); some of these long forms measured as much as 32 μ. Dividing parasites were always found in the crop, less often in the stomach and mid-gut, and very rarely in the rectum. As mentioned before the bodies supposed to be the resting stages were found in the faeces on two occasions. In one Melon Bug the salivary glands were found to be swarming with parasites, many of which were dividing. Several of the drawings were made from parasites from these glands.

The Method of Infection.

Very little can be said on this point as infected bugs were extremely rare. Patton's view that the liquid faeces are sucked up by other bugs seems by far the most acceptable hypothesis regarding the mode of infection. As mentioned before a large number of larvae and nymphs were examined but they were never found to be infected. In the few infected bugs which were studied careful examination of the ovaries was made but no parasites were discovered.

Experiments.

An emulsion of the contents of the crop of a bug, which contained many flagellates, was made in salt solution and injected into a gerbil; the result was negative, no infection taking place. An attempt was made to keep the flagellates in citrate of soda solution but they all died quickly.

Concluding Remarks.

Herpetomonas aspongopi is a true parasite of *Aspongopus viduatus*, the complete cycle of development taking place in the alimentary tract of the bug. The parasite is not transmitted hereditarily, but it probably

is conveyed from host to host by the ingestion of faeces containing resting forms. No parasites were observed in conjugation, nor could male or female forms be distinguished.

Patton has drawn attention to the resemblance between *Herpetomonas lygaei* and the Leishman-Donovan body, and this flagellate also offers a similar resemblance, consequently it belongs to the same category. The flagellate stage is distinguished by the formation of a typical flagellum, and by division and multiplication of the flagellates. The non-flagellate stages closely resemble the Leishman-Donovan body, the parasite in the resting stage dividing into two parasites, which afterwards develope into two flagellated forms.

For some time past there have been considerable differences of opinion concerning the ancestry of haemoflagellates. According to Minchin's view the haemoflagellates were originally parasites of the intestines of vertebrates, from whence they wandered into the blood stream, and were taken up later by bloodsucking insects, thus becoming parasites of a vertebrate and of an invertebrate host. This hypothesis applies to many forms of haemoflagellates, but, as Prowazek pointed out, *Herpetomonas muscae domesticae* is a true parasite of the house fly; this insect is not a bloodsucker although its ancestors may have been, as a study of the mouth parts of the house fly appears to indicate. *Herpetomonas aspongopi* is likewise a true parasite of *Aspongopus viduatus*, which is not a bloodsucker. Many of the Hemiptera are fierce bloodsuckers, as for example the bed bugs (*Acanthia*). The beak-like mouth-parts of the bed bugs bear a definite resemblance to the mouth-parts of the plant-feeding bugs, therefore it might be claimed that the ancestors of the latter were originally bloodsuckers, and that they had originally become infected with Herpetomonad forms from some vertebrate host.

I should like to thank Dr Balfour and Captain Archibald for their kind help and advice.

THEILERIA PARVA: ATTEMPTS AT CULTIVATION.

By GEORGE H. F. NUTTALL, F.R.S.,
and G. S. GRAHAM-SMITH, M.D.

In an earlier paper (1908, pp. 255—257)[1], we gave a full account of a paper by Miyajima (1907) wherein this author described experiments in which he states that he succeeded in cultivating *Theileria* (*Piroplasma*) *parva*. According to Miyajima, he had no difficulty in cultivating the parasite when he added the blood of cattle (containing *Theileria*) to ordinary bouillon in the proportion of 1 : 5 to 1 : 10, the cultures being maintained at 20—30° C. Miyajima states that trypanosomes appeared in his cultures after an interval of 3 days and underwent vigorous multiplication reaching "the maximum after the tenth to fourteenth day." Miyajima's description of the supposed process of development is obscure, and it is difficult to understand how the diminutive intra-corpuscular *Theileria* can develop into a *Trypanosoma*. We refer the reader who desires particulars regarding these experiments to our paper already cited. In our paper we stated that "a certain amount of scepticism" appeared justified until Miyajima's results had been extended and confirmed. The warning appears to have come too late in respect to Woodcock's recent review of the Haemoflagellates in Ray Lankester's *Treatise on Zoology* (1909, Part I. fasc. 1, p. 260), for Woodcock appears to accept Miyajima's remarkable discovery as authentic and conclusive.

Having, through the courtesy of Mr C. P. Lounsbury, Entomologist to the Department of Agriculture, Cape Colony, come into the possession of ticks (*Rhipicephalus evertsi*) infected with *Theileria parva*, we have recently been able to test the accuracy of Miyajima's cultivation

[1] Nuttall, G. H. F. and Graham-Smith, G. S. (1908). The development of *Piroplasma canis* in culture. *Parasitology*, I. 243—260, 1 text-figure, Plate XIX.

experiments. It must, however, be remembered that Miyajima conducted his experiments in Japan, whereas we conducted ours in England with parasites conveyed to cattle by means of ticks which were imported from South Africa. There can, however, be no doubt as to the similarity of the parasites with which we were dealing.

We have to report upon two carefully conducted experiments in which we failed to obtain results confirming those of Miyajima.

Cultures examined after the following number of days had elapsed	Mixtures consisting of		
	Blood 1 c.c., bouillon 2 c.c. to 10 c.c.	Blood 1 c.c., ·8 % NaCl, Solution 1 c.c.	Blood 1 c.c., 2 % citrate Solution 5 c.c.
1.	Corpuscles and Theileria normal.	Same as in bouillon.	—
2.	Corpuscles normal or crenated, some with normal Theileria. Large vacuolated granular leucocytes found in groups.	Corpuscles and Theileria normal.	—
3.	Corpuscles normal or crenated; Theileria mostly marginal or rounded, slight dancing movement; a few swollen leucocytes. When stained, some Theileria still take blue colour. Chromatin in parasites aggregated in masses.	Same as in bouillon. When stained, corpuscles appear shrunken, some parasites free, some marginal.	Same as in bouillon.
4.	Same as before.	Same as before.	Same as before.
5.	,, ,,	,, ,,	,, ,,
6.	,, ,,	,, ,,	,, ,,
12.	Corpuscles well preserved. Theileria mostly rounded and marginal. When stained, corpuscles appear pale, Theileria at times free; stain intensely with Giemsa but without trace of blue.	Corpuscles shrunken, slightly crenated; Theileria rounded, marginal. Stained appearances much the same as in bouillon.	Same as in bouillon.

EXP. I. In this experiment 30 ticks were placed on a cow on 10. II. 1909. On 6. III. an examination of the blood showed that 56 % of the red blood-corpuscles harboured *Th. parva*. Blood was now taken from a vein with aseptic precautions, and, after it had been defibrinated by being shaken for 25 minutes in sterile glass-stoppered bottles, measured quantities of the blood were mixed with measured quantities of bouillon, sodium citrate or sodium chloride solutions respectively. The sterile cotton-plugged vessels, containing the mixtures, were then incubated at 24° C. The vessels used were (*a*) ordinary test-tubes, and (*b*) small bottles such as we had used successfully for the cultivation of *Piroplasma canis*. The bouillon, which was approximately neutral,

answered Miyajima's description of "ordinary bouillon" (meat extract, peptone 1 %, NaCl ·5 %). The sodium citrate was used in 2 % solution in physiological salt solution (·8 %). The NaCl solution was ·8 %. The cow died of East Coast Fever on 13. III. 1909, that is, a week after the blood was withdrawn for cultural purposes.

The cultures were examined at various intervals of time in unstained and stained preparations made from the surface layer of corpuscles and leucocytes which had gravitated to the bottom of the vessels.

Exp. II. In this experiment 24 ticks, of the same lot as in Experiment I, were placed on a cow on 6—11. IV. 1909. On 25. IV. the parasites were detected for the first time in the cow's blood. On 26. IV. an enumeration showed that 6 % of the corpuscles harboured parasites. Venous blood was now collected in the same way as in the previous experiment. The cow died of East Coast Fever on 5. V. 1909.

The cultures were examined both fresh and stained. They were prepared as follows: (*a*) blood and bouillon as in experiment I, (*b*) blood and ·8 % NaCl solution mixed in the proportion 1 : 1 or 2 : 1, (*c*) blood and 4 % citrate mixed in the proportion 5 : 1. The results agreed entirely with those detailed under Experiment I.

Conclusions.

Our observations do not confirm those of Miyajima, since we never saw any development of *Theileria parva* in cultures. We have seen degenerated leucocytes assume various shapes which at times agreed in appearance with that of some of the developmental forms described by Miyajima. We conclude that the flagellates described by Miyajima as occurring in cultures of cattle blood containing *Th. parva* were probably cultural forms of a *Trypanosoma* which he failed to detect in blood smears taken from animals because of their small numbers in the circulating blood. Although we are at present only able to report upon two experiments, owing to our limited facilities for keeping cattle in the laboratories at Cambridge, we consider that they sufficiently indicate the need of caution in accepting Miyajima's results until further experiments are forthcoming.

NOTE ON ATTEMPTS TO INFECT THE FOX AND THE JACKAL WITH PIROPLASMA CANIS.

By GEORGE H. F. NUTTALL, F.R.S.,

and G. S. GRAHAM-SMITH, M.D.

All attempts hitherto made to infect different species of animals with *P. canis* have failed, and the remarkable fact remains that this parasite is apparently only communicable to dogs.

Robertson (1901, p. 332), in South Africa, obtained negative results when he attempted to transmit the disease by blood inoculations to horses, oxen, sheep, cats, rabbits, guinea-pigs, rats, mice and fowls. Similarly, Nocard and Motas (1902, p. 275), experimenting with the European strain of *P. canis*, failed to infect horses, oxen, sheep, goats, cats, rabbits, guinea-pigs, white mice, white rats, fowls or pigeons (see Nuttall, 1904, p. 245). Nuttall and Graham-Smith, experimenting with the South African strain of *P. canis* in Cambridge, failed to transmit the infection to cats, ferrets, hedgehogs, guinea-pigs and white rats.

Thinking that other Canidae might serve to maintain the parasite in nature, we decided to test various species of *Canis*, if the opportunity arose. The present note describes our experiments on foxes and Lounsbury's experiments on jackals in Cape Colony.

Experiments with Foxes, Canis vulpes L.[1]

The following experiments were carried out by us in Cambridge:

Experiment I.—Old Fox.

26. iv. 07. Received a subcutaneous injection of 40 drops of virulent blood taken from the ear vein of a dog with many parasites in its blood.

1. v. 07. Re-inoculated with 3 c.c. of defibrinated virulent blood.

[1] We are much indebted to Mr W. F. Cooper for kindly supplying us with the foxes.

18. v. 07. Result negative. No parasites found. The fox was killed and all the organs were carefully examined. Ten c.c. of the fox's heart-blood were injected into a dog, but the dog remained well.

Controls: Two dogs were inoculated with 4 c.c. each of the same samples of blood which were injected into the fox (26. IV. and 1. IV. 07). Both contracted fatal piroplasmosis.

Experiment II.—Young Fox.

15. v. 07. Received 3 c.c. of blood from the heart of a dog dead of piroplasmosis.

20. v. 07. Fox found dead; autopsy negative.

Control: A dog inoculated with 3 c.c. of the same blood which was injected into the fox, was killed on 20. v. 07 when moribund from piroplasmosis.

Experiments III, IV, V. Three young Foxes.

28. VII. 07. The foxes each received 4·5 c.c. of virulent blood.
The foxes remained well for over a month.
A dog which received an inoculation with the blood of Fox III on 27. VIII. remained free from piroplasmosis, but died from other causes a month later.

Control: A dog, inoculated with 2 c.c. of the same blood which was injected into the foxes, was killed on 1. VIII. when moribund from piroplasmosis.

Result: The four experiments prove that the fox is immune to South African piroplasmosis.

Experiments on the Jackal, Canis mesomelas Schreb.

Lounsbury, in his report as Entomologist to the Department of Agriculture, Cape of Good Hope (1903, p. 42), briefly states that he failed to infect a jackal either by inoculation or the application of ticks. Mr Lounsbury has kindly sent us the complete record of this experiment, together with the record of a second (unpublished) experiment, which was also negative, carried out in May, 1908.

The following is a summary of these experiments:

Experiment I.—Lasting from 30 Jan. to 20 Mar. 1903.

	Day	
(30. I. 03.)	1.	The jackal was infested heavily with pathogenic *Haemaphysalis leachi*. (Specimens of the same lot of ticks produced many cases of piroplasmosis in dogs without failures.)
	6.	Many ticks attached. Animal scratches at them a great deal.
	8.	3 partly gorged female ticks scratched or taken off.
	9.	12 gorged female ticks removed; 1 found loose in box.
	10.	about 20 ,, ,, ,,
	11.	about 6 ,, ,, ,,
		(Judging from the number of female ticks which were recovered, at least 80 males and females must have become attached to the jackal.)

23. Inoculated subcutaneously with 7 c.c. of virulent blood from Dog 68 (very sick).

34. Bled, and 7½ c.c. of the jackal's blood injected into Dog 76.
(The dog remained well for 24 days afterwards. Its temperature, taken twice daily, remained normal. The dog was not immune for pathogenic *H. leachi*, some were placed on it and it died of piroplasmosis on the 20th day after tick-infestation commenced.)

(20. III. 03.) 50. Jackal has up to the present shown no fever.

The jackal's temperature was taken once a day beginning with day 6, twice a day beginning with day 23, excepting on three days when it was taken once; on five days the temperature was not taken. The temperature remained normal throughout, varying from 100 to 103·2° F. The latter reading was only obtained once, two days after the inoculation with blood, otherwise the temperature usually varied between about 101·4 and 102·6° F.

Controls: see note under Day 1.

Experiment II.—The jackal used in this experiment was a young animal which had been brought to the experiment station about November, 1907. It was inoculated on January, 1908, with supposedly infected blood, but the blood was avirulent, since it failed to infect control dogs. A second experiment was made on this animal lasting from 6 May to 23 May, 1908.

	Day	Temperature, °F a.m.	p.m.	
(6. v. 08.)	1.	The jackal was inoculated with 10 c.c. of virulent blood (from a fatal case) diluted with citrate.		
	2.	104·4	102·2.	
	3.	104	103.	
	4.	103	103·6.	
	5.	103·2	103.	
	6.	103·2	102·2.	
	7.	102·6	104.	
	8.	102·6	103.	
	9.	103	104.	Jackal normal. No parasites in blood.
	10.	103	104·4.	
	11.	103	104.	Jackal quite normal.
	12.	102	103·2.	
	13.	103	103·4.	All hope of piroplasmosis infection abandoned.
	14.	103	104.	
	15.	102·6	101·6.	Given heavy dose of atoxyl chiefly to see if the
	16.	104	103.	jackal would survive it.
	17.	101	—	
(23. v. 08.)	18.	—	—	Died of atoxyl (arsenical) poisoning.

Controls: Three control dogs received 5 c.c. each of the same blood of which the jackal received 10 c.c. On the 9th day the dogs had developed fever, on the 11th two control dogs died. The record omits to mention what occurred in the case of the third dog, presumably it also died.

Result: These two experiments seem to indicate that jackals are immune to South African piroplasmosis. The possibility, however,

remains that the jackals had had the disease before they were captured and consequently that their immunity was acquired. Nevertheless, this possibility appears remote in the case of the second animal, which was captured as a young pup, and was maintained in captivity for six months before it was inoculated with virulent blood.

Conclusions.

The foregoing experiments appear to indicate that *P. canis* is peculiarly specific in its pathogenicity, since it is incapable of producing disease in the fox and the jackal, species closely allied to the dog. They also seem to show that neither of these species is concerned in the maintenance of the disease in nature. Experiments on the wolf would be extremely interesting.

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NOTES ON IMMUNITY IN CANINE PIROPLASMOSIS.

By GEO. H. F. NUTTALL, F.R.S. and G. S. GRAHAM-SMITH, M.D.

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THE discovery by Nuttall and Hadwen (VI. 1909, This *Journal*, vol. II. p. 156) that trypanblau and trypanrot exert a curative effect upon canine piroplasmosis, leads us to publish the immunization experiments described in this paper, although they have not yielded promising results. We do not consider that further experiments of the kind are required since there is little evidence that they will lead to a practical means of protecting dogs against piroplasmosis. On the other hand the drug treatment referred to gives us a means of curing the animals, and, combined with previous inoculation with virulent blood, affords us a practical means of "salting" animals without there being much risk attached to the procedure.

1. (*a*) *Attempts at immunization by means of immune serum.* (*Earlier experiments by Nocard and Motas, and Robertson.*)

It will be remembered that Nocard and Motas (1902) reported upon experiments which indicated that immune substances are developed in the serum of dogs that have recovered from piroplasmosis. Their

results may be briefly summarized as follows: on mixing the blood of recovered dogs with virulent blood and injecting the mixture into healthy dogs the latter did not become infected. They attributed this result to the existence in the immune serum of a substance which they thought was capable of destroying the parasites. They stated that the germicidal substance was greatly increased in amount in the serum of hyper-immunized dogs, that the serum of recovered animals had marked protective properties when injected in large doses 24—48 hours before inoculation with virulent blood, and that the serum of hyper-immune animals was much more active in this respect since 3 to 5 c.c. sufficed to protect susceptible dogs. They found that immune serum still exerted a protective action after having been heated to 56—57° C. Infected red blood corpuscles, exposed to the action of immune serum, and afterwards washed and injected, also conferred immunity. Passive immunity only lasted a short time, for animals upon which passive immunity had been conferred could be infected after periods varying between 11 and 35 days. Finally they stated that immune serum was curative if injected before the parasites appeared in the blood of inoculated dogs.

The results of Robertson (1906) at the Cape are in marked contrast to those of Nocard and Motas in France. Robertson found that the parasites remained fully virulent for a considerable time in the blood of "recovered" dogs. When the blood of the two "salted" dogs he used *had ceased to be infective,* he tested it for immunizing power on fresh dogs. He injected doses of 20 c.c. of recovered blood into the dogs he desired to protect and then injected 3 c.c. of virulent blood. The results were uniformly negative.

When Robertson sought to hyper-immunize salted dog "11," by injecting 20 c.c. of virulent blood, the dog developed a mild attack of piroplasmosis (temperature 104° F. on the 5th day; parasites in the blood for over 14 days).

Salted dog "45" was injected 6 times subcutaneously at intervals of 3 weeks with virulent blood, the doses of blood amounting respectively to 10 c.c., 25 c.c., 50 c.c., 250 c.c., 500 c.c. and 1000 c.c. This dog reacted to each injection but did not suffer ill-effects from the disease although the parasites reappeared in its blood and persisted therein for 9 days. Twenty-seven days after the last injection of virulent blood the dog was bled and 500 c.c. of its serum were collected. The serum was tested 16 hours later as follows:

Nine dogs were treated with the immune serum: 2 received 5 c.c. each and 7 received 10 c.c. each injected subcutaneously. In accordance

with the mode of treatment the experimental dogs may be grouped in 4 categories as follows: (*a*) four dogs received the immune serum 14 days before they were inoculated with virulent blood. (*b*) Two dogs received injections of immune serum simultaneously with the injections of virulent blood; in the one case the latter was mixed with the immune serum prior to injection, in the other case the injections were made separately on opposite sides of the dog's body. (*c*) One dog received the injection of immune serum five days after inoculation. (*d*) Two dogs received injections of immune serum every day after they showed fever following upon inoculation with virulent blood.

All of the nine dogs died, but in the case of the last two (*d*) no parasites could be detected in their blood at death. Robertson remarks that the results of his treatment by means of immune serum did not appear promising. He refers to the *absence of parasites* in the two dogs (*d*) as being of interest.

This observation certainly does possess interest since we have always found parasites in the blood of dogs dying from the disease. We have only observed two analogous instances:

(1) In a dog which was treated with trypanrot when in an advanced stage of piroplasmosis; in this case the parasites disappeared on the 5th day after treatment and could not be found in the blood subsequently. (Nuttall and Hadwen, Dog 2, p. 174, This *Journal.*)

(2) In Dog 1 (see p. 219), reported in this paper, which we sought to render immune by preliminary treatment with blood containing dead parasites. In both of these dogs parasites had been previously detected in the blood. Robertson does not state whether parasites were present in the blood of his two dogs at an earlier stage but presumably they were present since he notes that the dogs had fever. It appears from these cases as if a partial immunity had been attained, but the experiments are too few in number to be conclusive. Moreover, if the dogs had survived longer the parasites might have reappeared after the manner observed in the dogs treated by Nuttall and Hadwen by means of drugs.

(See Summary (1) on p. 225.)

Owing to the limited facilities for keeping dogs in our Cambridge laboratories it has been found impossible to repeat the experiments by Nocard and Motas, and Robertson. We however carried out other experiments in the hope that they might lead to results possessing practical value. We have been disappointed in this hope, and see no

reason for pursuing the investigations in this direction after the excellent results from drug treatment obtained by Nuttall and Hadwen.

(*b*) *Authors' attempts at immunization by inoculations with blood containing dead parasites.*

In the experiments about to be described an attempt was made to render dogs immune to piroplasmosis by a method which has not been attempted hitherto. We sought to render dogs immune by injecting them with successive doses of blood containing dead parasites. The blood used for immunization was collected from the hearts of dogs killed in an advanced stage of the disease so as to ensure the presence of many parasites in it. We hoped that this injection of dead parasites into the dogs might lead to the formation of protective antibodies in the animals. To ensure the death of the parasites the blood was either allowed to dry or it was allowed to stand at room temperature after the addition of a small amount of citrate and of carbolic acid.

Only one experiment (Dog 5) was carried out with blood which had been dried because it was found difficult to redissolve dried blood completely and aseptically.

The fluid blood, used for treatment, was defibrinated and citrate was added with the object of preventing the secondary coagulation which occasionally takes place. Measured quantities of blood (usually 10 c.c.) were placed in sterile cotton-plugged test-tubes after which 3 to 5 drops of 5 % carbolic acid solution were added to each tube. After a short time the corpuscles gravitated to the bottom of the tubes. In some cases the whole of the fluid in the tubes was shaken up and injected, in other cases the supernatant fluid was pipetted off and a concentrated suspension of corpuscles was injected.

The fluid blood, used for treatment, was usually allowed to stand for several days before it was injected, for in two cases the parasites survived in a virulent state in the weakly carbolized blood for periods of 24 and 72 hours respectively at room temperature. In all cases the dogs received several injections of blood prior to their being subjected to the test inoculation with fresh virulent blood.

The following protocols relate to four dogs which were treated with fluid blood and to one dog which was treated with dried blood. All the dogs died. Dog 1 showed parasites on the 10th day after inoculation with virulent blood but no parasites could be detected in its heart-blood at autopsy (partial immunity?, see p. 222). Dogs 2, 3 and 5 died of piro-

plasmosis with many parasites in their blood. The history of Dog 4 is given in considerable detail, the blood having been examined daily by Nuttall and Hadwen. Methylene blue was injected subcutaneously on the 8th day after inoculation, but the dye exerted no effect upon the parasites. On the 9th day 14 % of the corpuscles were found to be infected. Subsequently the number of infected corpuscles decreased but the dog showed severe symptoms. Parasites could not be detected in blood films on days 15, 18, 19 and 21—28. The parasites then reappeared and the dog finally succumbed to the chronic form of piroplasmosis.

Inoculations with fluid blood containing dead parasites.

Dog 1.

(Puppy), received 8 subcutaneous injections of citrated blood obtained from three dogs which died of piroplasmosis. The amounts of blood injected (deducting the citrate solution with which it was mixed) were as follows:

Doses of blood in c.c. =	5	2·5	2·5	5·3	2·2	6·6	6·6	6·6	= Total 42 c.c.
Number of days the blood had been kept in vitro	5	6	7	6	8	10	15	3	
Intervals between injections, in days	2	2	10	2	2	5	5		

Ten days after the last injection the dog received 3 c.c. of virulent blood.

Result: 10 days after inoculation a very few parasites were found in the dog's blood and 8 days later (18 days after inoculation) the dog died during the night from no apparent cause. The dog had not shown haemoglobinuria and no parasites could be found in its heart-blood at autopsy.

Dog 2.

(Large Terrier), received 4 subcutaneous injections of concentrated suspensions of infected corpuscles (sedimented in citrate solution containing a few drops of carbolic acid). The amounts injected are reckoned in terms of ordinary blood.

Doses of blood in c.c. = ...	10	10	4	15	= Total 39 c.c.
Number of days the blood had been in vitro	8	10	12	15	
Intervals between injections, in days ...	2	2	3		

A week after the last injection the dog was inoculated with virulent blood.

Result: The dog died of piroplasmosis after 10 days.

Dog 3.

(Terrier, adult) was treated like Dog 2. It received 5 injections; the amounts injected being reckoned in terms of ordinary blood.

Doses of blood in c.c. = ...	10	18	20	18	20	= Total 86 c.c.
Number of days the blood had been in vitro	8	9	12	8	10	
Intervals between injections, in days ...	13	3	3	2		

Nine days after the last injection the dog received 5 c.c. of virulent blood.

Result: Parasites appeared in the dog's blood on the 6th day after inoculation and the dog died two days later (8th day after inoculation) of piroplasmosis. The dog had haemoglobinuria and there were many parasites in its blood, 29 % of the corpuscles being infected. The types of parasites encountered in the blood on the day when the dog died were as follows: 40 % (O), 52 % (PP), 3 % (OO), 3 % (PPPP), 2 % (D)[1].

[1] The signs used in this paper are similar to those used in the paper by Nuttall and Hadwen (1909, p. 162, q.v.).

Dog 4.

(Adult Terrier) was treated like Dog 3 and at the same time. The dog received 6 injections of blood prior to inoculation.

Doses of blood in c.c. = ...	10	9	20	20	10	20 = Total 89 c.c.
Number of days the blood had been in vitro	8	10	9	12	8	10
Intervals between injections, in days ...	2	11	2	3	2	

(15. IV. 09)

Day

1 Nine days after the last injection the dog received 5 c.c. of virulent blood containing many parasites.

Day	Temp. °F.		Infected r.b.c.	Percentages of P	D	O	PP	OO	DD	PPO	P_4	P_6	P_8
6	103·0	Parasites appeared in dog's blood	+	·	·	·	·	·	·	·	·	·	·
7	104·6	25 infected r.b.c. at film-edge ...	+	·	·	60	36	·	·	·	4	·	·
8	106·0	a.m. Treatment: 6 c.c. of 1 % methylene blue (exerted no visible effect on parasites)	5	2	·	49	42	3	·	·	4	·	·
		p.m.	–	·	1	48	44	·	·	·	7	·	·
9	105·0	... ...	14	2	·	45	48	1	·	·	4	·	·
10	104·2	Dog very weak; haemoglobinuria	5	1	1	47	50	·	1	·	·	·	·
11	103·8	Distinct anaemia	2	4	·	50	42	·	·	·	4	·	·
12	101·0	Few parasites found	+	·	·	+	+	·	·	·	·	·	·
13	101·2	Dog weak, anaemic. (No less than ⅙th of all parasites seen were free pyriforms, many corpuscles breaking up)	0·8	28	·	38	26	·	·	·	8	·	·
14	103·4	Dog very weak and anaemic. Only 4 infected r.b.c. found after long search: 2 (O), 1 (PP), 1 (PPPP). Occasional bacilli in film ...	+	·	·	+	+	·	·	·	+	·	·
15	104·3	Condition as on previous day; no infected r.b.c. found ...	·	·	·	·	·	·	·	·	·	·	·
16	103·5	Dog exceedingly weak, gait tottering, 4 infected r.b.c. found, 3 (PP), 1 (PPPP)	+	·	·	·	+	·	·	·	+	·	·
17	102·8	Condition unchanged, blood watery, 3 infected r.b.c. found: 3 (OO) ...	+	·	·	·	·	+	·	·	·	·	·
18	101·3	Condition slightly improved. No infected r.b.c.	·	·	·	·	·	·	·	·	·	·	·
19	102·4	Dog better, feeding, no infected r.b.c.	·	·	·	·	·	·	·	·	·	·	·
20	104·8	Dog stronger, 1 infected r.b.c., (O)	+	·	·	+	·	·	·	·	·	·	·

21	104·0	Dog stronger, no infected r.b.c. ...	·	·	·	·	·	·	·	·	·	·	·
22	104·8	,, ,, ,, ,, ,, ...	·	·	·	·	·	·	·	·	·	·	·
23	102·2	Blood more normal, no infected r.b.c.	·	·	·	·	·	·	·	·	·	·	·
24	101·4	No infected r.b.c.	·	·	·	·	·	·	·	·	·	·	·
25	100·1	,, ,, ,, ...	·	·	·	·	·	·	·	·	·	·	·
26	101·7	No parasites	·	·	·	·	·	·	·	·	·	·	·
27	101·8	,, ,, ...	·	·	·	·	·	·	·	·	·	·	·
28	103·6	,, ,, ...	·	·	·	·	·	·	·	·	·	·	·
29	105·2	Parasites reappear	0·4	2	·	32	34	10	·	·	18	2	2
30	105·4	Dog ill. Blood count:											
		a.m. (200 infected r.b.c. recorded)	1	·	1	22·5	32	4	1·5	0·5	28·5	0·5	9·5
		p.m., sudden fall of infected r.b.c., only 11 found on whole film-edge: 6 (O), 1 (PP), 4 (PPPP)	+	·	·	+	+	·	·	·	+	·	·
31	104·8	4 infected r.b.c. found on whole film-edge: 2 (O), 1 (PP), 1 (PPPP)...	+	·	·	+	+	·	·	·	+	·	·
32	102·4	6 infected r.b.c. on film-edge: 3 (O), 3 (PP)	+	·	·	+	+	·	·	·	·	·	·
33	104·2	Parasites in larger numbers ...	2	5	1	31	46	2	·	·	15	·	·
34	103·0	,, ,, ,, ...	1	7	1	37	51	·	·	·	4	·	·
35	103·0 (20 v. 09)	,, ,, ,, ...	0·5	10	·	50	36	2	·	2	·	·	·
36	—	Dog found dead. Death due to the chronic form of piroplasmosis.											

Note:—The signs P_4, P_6, P_8 indicate corpuscles which contained 4, 6, and 8 pyriform parasites respectively.

Experiment with dried blood.

The blood used for the preliminary treatment of the dog in the following experiment was dried in quantities of 10 c.c. for 5 days at 35° C. The blood was subsequently kept at room temperature. For purposes of injection as much of the dried blood as possible was dissolved or brought into suspension in water by being rubbed up in a mortar or by being shaken up in sterile bottles containing glass beads. The grumous looking fluid was then injected subcutaneously.

Dog 5.

(Whippet) received 4 injections of blood solution.

Doses of blood in c.c. = ...	8	8	8	8	= Total 32 c.c.
Number of days the blood had been dried	6	9	11	13	
Intervals between injections, in days ...	3	2	2		

Nine days after the last injection the dog received 4 c.c. of virulent blood.

Result: Seven days after inoculation parasites appeared in the dog's blood and the animal subsequently died of piroplasmosis.

(See Summary (2) on p. 227.)

2. *The duration of "immunity" following "recovery."*

Whilst dealing with the subject of immunity in canine piroplasmosis it appears to us advisable to include a discussion on certain problems which have arrested our attention in the course of our investigations. We shall first consider the matter of the partial immunity which follows upon the subsidence of the acute manifestations of piroplasmosis, a condition in which the animals are referred to as "salted" or "recovered."

Both in the European and African disease immunity is stated to last for some time after recovery. Lounsbury (1901, p. 11) believes that under natural conditions the acquired immunity is maintained by "continuous infestation by ticks." According to Nocard and Motas (1902, p. 277) naturally acquired immunity and that following the disease induced by inoculation lasts as long as 6 months in the European disease. Robertson (VI. 1906, p. 113) states that under natural conditions one attack does not protect for life and that dog owners at the Cape state that dogs may have three or four attacks. In Robertson's experience one attack usually "permanently salts" a dog, but he has seen four cases in which dogs recovered one season and died from contracting the disease in the following season. This author has observed an instance, unique in his experience, where a dog acquired the disease three weeks after it had passed through a severe attack and its temperature had returned to normal. The second attack followed the injection of 5 c.c. of virulent blood and the dog succumbed to the infection. He has on the other hand proved that salted animals may resist blood inoculation and tick infection for a considerable period. Lounsbury (1901, p. 11) reports the case of a dog which died from the disease, having suffered from it two years previously.

It is difficult to judge of the value of these statements as the term immunity is usually employed in a loose sense. Properly speaking, an animal can only be described as immune when it no longer harbours a parasite and is at the same time resistant to reinfection with the same species of parasite. It is clear from what we note below with regard to the persistence of the parasites in the blood of apparently recovered or "salted" dogs, that animals are frequently termed immune when, strictly speaking, they are suffering from chronic piroplasmosis, from which it is true they may ultimately recover. It is possible, as Lounsbury suggests, that continuous reinfection through the agency of ticks maintains the acquired "immunity" since when reinfection ceases and the parasites disappear the animal again becomes susceptible. In other

words the chronic form of the disease as a rule protects against the acute form of piroplasmosis. We have no experimental evidence that a true immunity follows upon complete recovery from piroplasmosis.

(See Summary (3) on p. 227.)

3. *The persistence of parasites in apparently recovered dogs.*

That *Piroplasma canis* may persist for a considerable time in salted dogs, or those which have apparently recovered, has been amply proved. Thus Robertson (1902, p. 682, fully cited by Nuttall, 1904, p. 246) observed that a salted dog's blood remained virulent for $4\frac{1}{3}$ months after recovery (4 Nov. 1901 to 12 March 1902). He also reports a case (1906, p. 110, Dog 11) in which a dog's blood remained virulent for other dogs for a period of two years (17 Nov. 1901 to 4 Dec. 1903) and adds, "all this time the animal had been kept in a run where it was free from any infective influence." This dog's blood was tested for virulence on seventeen occasions during the two years, and in every case inoculation practised with its blood produced fatal piroplasmosis in fresh dogs. The dog's blood proved no longer infective on 11 Feb. 1904. In the case of another dog (No. 45) the blood remained infective for 13 months after apparent recovery.

In the only two instances in which we have had an opportunity of observing the occurrence of spontaneous or natural recovery in dogs infected with the South African disease the blood of the apparently recovered animals remained infective for periods of four and six months respectively but not longer. After these periods the dogs could be regarded as recovered animals in the strict sense.

In all of the above cases the blood of the dogs remained fully virulent as long as it remained infective, for it produced an acute and fatal disease when it was injected into fresh dogs. In other words there is no evidence that the parasite which causes South African piroplasmosis in dogs becomes in any way modified in its virulence in animals suffering from the chronic type of the disease.

Robertson's (1906, p. 113) statement that keepers of hounds or packs of dogs believe "that if an animal recovers from malignant jaundice he should be housed apart from his fellows for the rest of the season," affords a piece of collateral evidence that animals which are recovering from piroplasmosis are a potential source of danger to others.

The facts above cited are in direct contradiction to the statement of Nocard and Motas (1902, p. 273), but it must be remembered that they

The passage of P. canis through a series (I) of Dogs.

Dog No.	Amount of piroplasma blood injected	Day after inoculation when Parasites (p) or fever (f) were first noted	Day after inoculation when The dog was killed or died of the disease	Remarks
1	—	—	—	Tick infection. Killed 25. VII. 06, 11 days after onset of fever.
2	5 c.c.	6 f	13	Killed when moribund.
3	2	6 f	13	Died during night of day 12—13.
4	10	6 f	10	Killed, very ill.
5	10	5 f	8	,, ,,
6	10	—	9	Died, had shown little or no fever. Many parasites and haemoglobinuria present. (Blood and clots taken from heart at autopsy for inoculation into next dog.)
7	5	5 f	13	Died (Collie puppy). Blood for inoculation of next dog taken at autopsy.
8	4	10 f	13	Killed. (Adult dog.)
9	8	7 f	9	Killed, few parasites present (adult dog).
10	7	5 f	—	Parasites disappeared 6th day, *chronic case* (adult Collie).
11′	1 from ear vein	10 f	13	Died. Haemoglobinuria (young dog).
12′	—	—	13	Died. Haemoglobinuria (young dog).
13′	7 c.c.	9 p	13	,, ,, ,,
14′	4	7 p	12	Died. (Puppy $\frac{3}{4}$ yr. old.)
15′	20 drops from ear vein	7 p	9	Killed. (Puppy $\frac{1}{3}$ yr. old.)
16′	2 c.c.	10 f	11	Died. (Puppy $\frac{1}{2}$ yr. old.)
17′	4	11 f	11	Killed. (Puppy.)
18′	4	5 f	7	,,
19′	5	2 f	4	,,
20′	5	4 p, 5 f	8	,,
21′	5	—	17	Died. *No fever*, parasites found in liver smear.
11	45 drops from ear vein	10 p	17	Died, haemoglobinuria. The chronic dog's blood hence fully virulent 135 days after it had been inoculated.
12	5 c.c.	8 p, 10 f	11	Died.
13	6	3 f	8	Killed.
14	4	7 f	7	,,
15	5	2 f	6	,,
16	5	6 f, 7 p	9	,,
17	4	4 f	4	,,
18	4	3 f	5	,, Many parasites.
19	4	6 f	6	,,
20	6	4 f	5	,,
21	3	5 f	5	,,
22	3	6 f	7	,,
23	4	2 f	5	,,
24	3	6 f	6	,,
25	3	5 f, 6 p	7	,,
26	2	6 f	7	,,
27	3	—	8	,, No fever.
28	3	6 f	6	,,
29	2	4 f	5	,,
30	2	5 f	6	,,
31	4	8 p, 9 f	9	,,
32	3	2 f, 5 p	6	,,
33	4·5	2 f & p	4	,,
34	2	4 p, 5 f	11	,,
35	2	5 f & p	7	,,
36	2	4 f	5	,,
37	2	2 f, 6 p	7	,,
38	2	3 f & p	4	,,
39	2	10 p, 13 f	17	Found dead, few parasites found on 10th day, blood taken 13th day for inoculation of next dog.
40	42 drops of ear blood	—	—	Remained well. Reinoculated from Dog 10 (chronic case, 1 year after it was inoculated, but result negative).

experimented with the European form of canine piroplasmosis which we regard as an altogether milder affection than the South African. Nocard and Motas state that the blood of dogs suffering from the chronic form of piroplasmosis is less virulent than the blood taken from acute cases.

(See Summary (4) on p. 227.)

4. *Records relating to the passage of P. canis through series of Dogs.*

Series I of Dogs infected during 1906, 1907. (p. 224.)

The first dog was infected by means of ticks (*Haemaphysalis leachi*, 3 ♂ and 8 ♀) received from Mr Lounsbury, Government Entomologist, Cape Colony. The strain was subsequently maintained by inoculation of blood carried on from dog to dog as indicated by the brackets connecting the numbers of the dogs in the left-hand column. Excepting in a few cases (noted in the second column) where the blood used for inoculation was taken from a punctured ear vein during life, the inoculations were made with defibrinated heart-blood injected subcutaneously.

Series II of Dogs infected during 1908, 1909. (p. 226.)

This series of dogs was inoculated with the same strain of *P. canis* as Series I. The strain being recovered from Sir John MacFadyean to whom it had been sent and who maintained it by passage from dog to dog in London. Dog 1, in the following series, was inoculated in London and its blood served to start the new series in Cambridge.

SUMMARY AND CONCLUSIONS.

1. *Regarding attempts at immunization by means of immune serum.* The experiments of Nocard and Motas indicated that protective substances occur in the blood of dogs which have recovered from European piroplasmosis. These authors claimed that immune serum destroys the parasites and that it exerts a protective and curative effect upon the disease. Similar experiments conducted by Robertson in Cape Colony gave contrary results to those of Nocard and Motas. In the absence of further evidence no conclusions can therefore be drawn from these conflicting results. Two of Robertson's dogs which received injections of hyperimmune serum every day after they showed fever,

The passage of P. canis through a series (II) of Dogs.

Dog No.	Amount of piroplasma blood injected	Day after inoculation when: Parasites (p) or fever (f) were first noted	Day after inoculation when: The dog was killed (K) or died (D) of the disease	Remarks
1	—	8 f	9 K	Haemoglobinuria, many parasites. (Fox-terrier adult.)
2	4 c.c.	3 p, 6 f	6 K	Ditto.
3	5	5 p, 7 f	8 K	Ditto.
4	6	2 p, 2 f	6 K	Ditto (1 year old mongrel).
5	4	6 p, 8 f	10 K	Ditto.
6	5	4 p	8 K	Ditto (puppy, showed no fever).
7	5	6 p, 8 f	9 K	—
8	3	—	6 D	Blood taken at autopsy for inoculation into next dog was mostly serum (old dog).
9	3	5 p, 9 f	13 K	No haemoglobinuria, few parasites (puppy).
10	5	6 p, 8 f	9 K	Many parasites (puppy).
11	4	3 p, 4 f	7 K	Ditto (old Fox-terrier).
12	4	8 f	9 K	Ditto.
13	3	4 p	7 K	Ditto. No fever up to 6th day (puppy).
14	3	3 p, 5 f	6 K	Ditto (puppy).
15	4	6 p, 8 f	10 K	Ditto (Whippet).
16	4	4 p, 5 f	6 D	Ditto, haemoglobinuria (puppy).
17	7	4 p	7 K	Ditto.
18	5	7 p	4 K	Few parasites.
19′	5	7 p	8 D	Haemoglobinuria, Icterus.
19	5	—	8 K	Ditto, many parasites (old Fox-terrier).
20′	4	—	8 D	Many parasites (puppy).
20	4	—	5 K	Ditto.
21	5	5	7 K	Puppy.
22	5	4	5 K	Puppy, haemoglobinuria, 50 % r.b.c. infected.
23	4	8	9 K	Puppy, haemoglobinuria, many parasites.
24	4	6	10 K	Puppy, many parasites.
25	4	—	5 K	Old Fox-terrier, haemoglobinuria, many parasites.
26	4	6	7 K	Puppy, ditto.
27	5	3	6 K	Puppy, ditto.
28	4	4	6 K	Puppy, ditto.
29	4	8	12 K	Old dog.
30	5	8	11 K	Old Terrier.
31	4	—	9 K	Puppy, haemoglobinuria.
32	4	—	8 K	,,
33	4	5	7 K	,,
34	4	4	6 K	,,
35	4	4	6 K	,, many parasites.
36′	4	—	7 K	,,
37′	4	—	8 D	,, blood collected at autopsy for inoculation of next dog.
38′	4	—	15 D	,, many parasites.
36	5	—	8 —	,,
37	4	8	8 —	,,
38	60 drops	—	14 K	,, inoculated with blood from ear vein.
39	4 c.c.	—	13 K	Killed soon after parasites appeared.
40	5	—	13 K	Adult Terrier, had many parasites, haemoglobinuria, moribund when killed.
41	5	—	14 K	Adult terrier, moribund when killed.
42	5	—	10 D	Blood collected at autopsy to inoculate next dog.
43	4	—	8 K	
44	5	5	8 D	Adult dog, many parasites.
45	4	8	13 K	Young Collie, haemoglobinuria, 10 parasites per field, killed when moribund.
46	7	3	7 K	Adult Terrier, ditto.
47	5	4	7 K	Large Collie, killed when moribund and 28 % of r.b.c. infected.
48	6	—	9 D	Adult Terrier, haemoglobinuria, many parasites.

died, but no parasites could be found in their blood at death. This result suggests that a partial immunity may have been obtained by the treatment.

2. *Regarding attempts at immunization by means of inoculations with blood containing dead parasites.* Of the five dogs which we attempted to immunize in the manner we have described, three died of acute piroplasmosis; one died without parasites in its blood although parasites had been previously found; one dog died on the 36th day from chronic piroplasmosis. The absence of parasites in the blood of one dog at autopsy and the occurrence of chronic piroplasmosis in another dog may or may not indicate a partial acquisition of immunity. In any case the experiments afford no evidence that practical results are likely to follow further investigation of this character.

3. *The duration of immunity following recovery* is undetermined since there are no experiments to prove that animals are immune after the parasites have completely disappeared from their blood. In the so-called "immune," "recovered," or "salted" dogs, the animals harbour parasites in small numbers for weeks, months or years, consequently such dogs are suffering from a mild chronic form of piroplasmosis. Whilst subject to this mild form of the disease dogs usually escape acute infection when reinoculated with the parasite.

4. The parasites of piroplasmosis canis may persist in the blood of apparently recovered dogs for a considerable length of time, 6 months to 2 years, and so long as they are present in the blood the latter remains fully virulent for clean dogs. Consequently there is no evidence that the African *Piroplasma canis* becomes modified in its virulence during the course of chronic piroplasmosis. According to Nocard and Motas the European *P. canis* does become modified in its virulence in dogs suffering from chronic piroplasmosis. It should be noted that the European disease is a milder affection than the African.

5. The passage of the African *P. canis* through two series of dogs, that is through upwards of 90 animals in the course of two and a half years, has shown that the parasite may be communicated by inoculation from dog to dog for an indefinite period. No evidence was obtained that *P. canis* is in any way modified in its virulence by passage through dogs. In a number of dogs which were inoculated with post-mortem blood the disease developed more slowly than usual but nevertheless led to a fatal issue.

6. The onset of fever after inoculation with virulent blood may precede or succeed the appearance of *P. canis* in the peripheral circula-

tion. Occasionally the disease may run its course to a fatal termination without the appearance of febrile symptoms.

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FURTHER EXPERIMENTS UPON THE DRUG TREATMENT OF CANINE PIROPLASMOSIS

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Preventive Treatment with Trypanblau.

In our previous paper (vi. 1909, p. 187) we described an experiment in which a dog (No. 13) was inoculated with virulent blood and on the day following received an injection of trypanblau subcutaneously. This dog showed no parasites, although its blood was examined almost daily up to the 49th day after inoculation. A control dog, on the other hand, died on the 7th day after inoculation.

The result of this experiment was so striking that we determined to repeat it, using a number of dogs for the purpose so as to exclude any possible error.

The following experiments were carried out on nine dogs, all of which were inoculated (23. iv. 1909) with defibrinated heart-blood taken from a dog which was killed whilst moribund from piroplasmosis. Two of the nine dogs served as controls.

Whereas six of the dogs received a dose of Trypanblau on the day [" 0 "] before they were inoculated, one dog received the dose on the day after inoculation. All of the dogs were inoculated subcutaneously. The treated dogs were injected subcutaneously with the dye in cold saturated solution.

Treated Dogs.

Dog	Day	
1	0	*Treatment:* 2 c.c. of trypanblau solution.
	1	*Inoculated* with 3 c.c. of virulent blood.
	22	No parasites found to date, dog well.
2	0	*Treatment:* 2 c.c. of trypanblau solution.
	1	*Inoculated* with 3 c.c. of virulent blood.
	26	No parasites found to date, dog well.
3	0	*Treatment:* 1·8 c.c. of trypanblau solution.
	1	*Inoculated* with 3 c.c. of virulent blood.
	23	No parasites found to date, dog well.
4	0	*Treatment:* 2·3 c.c. of trypanblau solution.
	1	*Inoculated* with 3 c.c. of virulent blood.
	30	No parasites found to date, dog well.
5	0	*Treatment:* 2 c.c. of trypanblau solution.
	1	*Inoculated* with 3 c.c. of virulent blood.
	27	No parasites found to date, dog well.
6	0	*Treatment:* 2 c.c. of trypanblau solution.
	1	*Inoculated* with 3 c.c. of virulent blood.
	28	No parasites found to date, dog well.
7	1	*Inoculated* with 5 c.c. of virulent blood.
	2	*Treatment:* 15 c.c. of trypanblau solution (large dog).
	28	No parasites found to date, dog well.

Control Dogs.

Dog	Day	
A.	1	*Inoculated* with 3 c.c. of virulent blood.
	7	*Parasites appeared.*
	10	*Dog died* of piroplasmosis with many parasites in its blood.
B.	1	*Inoculated* with 3 c.c. of virulent blood.
	23	No parasites to date, dog well.

As will be seen from the foregoing protocols, none of the seven dogs which received Trypanblau developed piroplasmosis. At no time were parasites detected in their blood, although examinations were made up to the 22nd and to the 30th day after inoculation. On the other hand, one

of the two Control Dogs (A) died of piroplasmosis on the 10th day after inoculation. Control Dog B remained well up to the 23rd day after inoculation, thus in a measure vitiating the experiment. We nevertheless consider that the result confirms the experiment on Dog 13, reported in our previous paper, and cited on p. 229, for the following reasons. It is a relatively rare occurrence for an inoculation to fail to produce infection. In our previous paper we recorded 25 consecutive inoculations, in which only one (Dog 12, p. 186) failed to give a positive result by the 11th day, when the dog was reinoculated. The only dog which did not show parasites was Dog 13. Again, all of the five dogs used in the remaining experiments described in this paper died from piroplasmosis as the result of inoculation. We conclude therefore that *Trypanblau injected a day before or a day after inoculation prevents the development of the disease by destroying the parasites.*

It is not quite certain upon what the negative result of the inoculation depended in the case of Dog B. In the few cases observed by one of us (N.), in the course of the last five years, where inoculations have failed to infect, the negative result could not be attributed to the existence of any immunity on the part of the dog. Upon reinoculation such dogs have invariably acquired the disease. We are inclined to believe that the negative result is attributable to mechanical causes, the injected blood remaining confined to the subcutaneous or cellular tissue at the seat of inoculation. Under such conditions the parasites cannot gain access to the blood corpuscles of the host, because they remain enclosed within an impervious limiting membrane, at any rate for a sufficient length of time to cause the parasites to perish from lack of fresh corpuscles, into which they must soon penetrate if they are to survive. The matter is not without interest and deserves investigation. To render infection certain, on the assumption that our explanation holds good, all that appears necessary is to massage the seat of the blood injection so as to prevent the blood from remaining confined as it were in a bag.

Experiments with Trypanblau given by the Mouth.

Owing to the objection on the part of owners of cattle against modes of treatment which necessitate the use of a syringe whereby remedies are injected either subcutaneously or intravenously, it appeared to us expedient to try if trypanblau exerted any effect upon the parasites when the dye was given by the mouth.

Judging from previous experience, we consider the presumption justified that experiments of this nature, conducted upon dogs, will give identical results in cattle.

In the following experiments both dogs (Irish terriers) were given Trypanblau in capsules per os beginning on the day following that on which they were inoculated.

Dog 1.

Date	Day	Temp. °F.		% infected r.b.c.	% (P)	% (O)	% (PP)	% (D)	% (PPPP)	% (OO)
(2. VII. 09.)	1.	—	5.30 p.m. *Inoculated* with 4 c.c. of virulent blood.							
	2.	—	12.30 p.m. *Treatment:* ¼ gram. of Trypanblau.							
	3.	—	11.30 a.m. ,, ,, ,, ,, Blue colour of skin observed.							
	4.	—	3.30 p.m. ,, ,, ,, ,,							
	5.	—	11 a.m. No parasites found.							
		102·6	5 p.m. *Treatment:* ½ gram. of Trypanblau.							
	6.	102·4	11.30 a.m. A few *parasites appeared* ...	·03	4	40	40	4	12	·
		102·3	6.15 p.m. dog looking very well.							
	7.	104·7	11 a.m. dog looks well.							
		104·0	6 p.m. ,, ,, ...	0·4	·	53	41	2	4	·
	8.	104·5	12.10 p.m. dog looks fairly well	14·6	4	52	37	2	4	1
(10. VII. 09.)	9.	—	9 a.m. dog *found dead.* Death due to piroplasmosis.							

Dog 2.

Date	Day	Temp. °F.		% infected r.b.c.	% (P)	% (O)	% (PP)	% (D)	% (PPPP)	% (OO)
(2. VII. 09.)	1.	—	5.30 p.m. *Inoculated* with 4 c.c. of virulent blood.							
	2.	—	2.30 p.m. *Treatment:* ¼ gram. of Trypanblau.							
	3.	—	11.30 a.m. ,, ,, ,, ,, Blue colour of skin apparent.							
	4.	—	3.30 p.m. ,, ,, ,, ,,							
	5.	—	11.30 a.m. *Parasites appeared*, only 3 infected r.b.c. found, 2(PP), 1(O)	+	?	+	+	?	?	·
		102·1	5 p.m. *Treatment:* ½ gram. of Trypanblau.							
	6.	102·6	11.30 a.m. dog looking well	·02	?	44	48	?	8	·
		101·7	6.15 p.m. ,, ,,							
	7.	102·6	11 a.m. dog looking well	1·6	·	49	47	3	1	·
		102·2	6 p.m. dog looking less lively.							
	8.	103·0	12 p.m. dog looks weak	6·4	1	48	45	3	3	2
(10. VII. 09.)	9.	—	10.30 a.m. *dog moribund*, severe haemoglobinuria, killed	28.0	2	47	43	3	3	2

It appears from the above experiments that Trypanblau, given by the mouth, is ineffective, and that it exerts no apparent influence upon the parasites.

Experiments with Tryparosan.

Owing to the excellent results recently obtained in the treatment of trypanosomiasis by means of Tryparosan in Ehrlich's laboratory (Roehl, XII. 1908; Marks, V. 1909) it appeared to us desirable to try the effect of this dye upon piroplasmosis in dogs. Tryparosan is prepared from parafuchsin by the addition of chlorine to the molecule. The dye is prepared by Messrs L. Cassella & Co., Frankfurt a M. We are much indebted to this firm for their courtesy in supplying us with the dye free of cost for the purposes of our experiments and take this occasion to warmly thank Geheimrath Ehrlich for the help he has given us in procuring this and other remedies in connection with our investigations.

In the following experiments both dogs (Irish terriers) were treated with Tryparosan. Dog 1 received a subcutaneous injection of a 1:500 solution of the dye on the afternoon of the day when the parasites first appeared. Dog 2 was given the dye in capsules per os (*a*) on the day following the inoculation; (*b*) on the day before, and (*c*) on the day the parasites appeared.

Dog 1. (*Subcutaneous Treatment.*)

Date	Day	Temp. °F.		% of infected r.b.c.	% (P)	% (O)	% (PP)	% (D)	% (PPPP)
(2. VII. 09.)	1.	—	5.30 p.m. *Inoculated* with 4 c.c. of virulent blood.						
	5.	—	11 a.m. *Parasites appeared*	+	•	•	•	•	•
		—	3.30 p.m. *Treatment:* 30 c.c. of Tryparosan solution. Oedema soon followed at site of injection	0·4	1	45	44	5	6
		103·8	5 p.m. Much oedema at site of injection ...	1·0	•	43	45	3	9
		104·4	9.30 p.m.	0·6	1	47	44	2	6
	6.	103·9	11.30 a.m.	15·0	•	45	51	3	1
		104·1	6.15 p.m. Dog looking ill. Much oedema.						
(8. VII. 09.)	7.	—	9 a.m. Dog *found dead*. Death due to piroplasmosis.						

Dog 2. (*Treatment by the mouth.*)

Date	Day	Temp °F.		% of infected r.b.c.	% (P)	% (O)	% (PP)	% (D)	% (PPPP)	% (OO)
(2. VII. 09.)	1.	—	5.30 p.m. *Inoculated* with 4 c.c. of virulent blood.							
	2.	—	12.30 p.m. *Treatment:* $\frac{1}{16}$ gram. Tryparosan.							
	3.	—	11.30 a.m. Pink colouration of skin noticeable.							
	4.	—	3.30 p.m. *Treatment:* $\frac{1}{16}$ gram. Tryparosan.							
	5.	—	11 a.m. *Parasites appeared:* 2 (PP) found	+	·	·	+	·	·	·
		102·2	5 p.m. *Treatment:* $\frac{1}{4}$ gram. Tryparosan.							
	6.	102·2	11.30 a.m. Few parasites	·05	·	40	40	4	12	4
		102·4	6.15 p.m. Dog looks well.							
	7.	104·2	11 a.m. Dog looks well	1·0	·	61	35	2	2	·
		104·1	6 p.m. ,, ,,							
	8.	104·8	12.15 p.m. Dog looks fairly well	18·0	3	52	37	2	6	·
(10. VII. 09.)	9.	—	9 a.m. Dog *found dead.* Death due to piroplasmosis.							

Control Experiments.

A Control Dog (Terrier) was inoculated at the same time as the four dogs referred to in the preceding two sets of protocols. The control dog received no treatment.

Date	Day	Temp. °F.		% of infected r.b.c.	% (P)	% (O)	% (PP)	% (D)	% (P_4)	% (P_5) to (P_8)
(2. VII. 09.)	1.	—	5.30 p.m. *Inoculated* with 4 c.c. of virulent blood.							
	5.	—	11.30 a.m. A few *parasites appeared*	+	·	·	·	·	·	
		103·3	5 p.m.							
	6.	103·9	11.30 a.m.	4	2	42	47	2	6	
		102·2	6.15 p.m. Dog looks ill.							
(8. VII. 09.)	7.	95·2	11 a.m. Dog moribund ...	88	·	23	36	·	28	14
		—	3 p.m. *Dog died* of piroplasmosis.							

Note:—The signs P_4, P_5, P_8 indicate corpuscles which contained 4, 5 to 8 pyriform parasites respectively.

It follows that Tryparosan exerts no influence upon *Piroplasma canis* and that it is ineffective as a remedy for canine piroplasmosis.

Summary and Conclusions.

1. Trypanblau injected subcutaneously into dogs a day before or a day after they have been inoculated with blood containing *Piroplasma canis* effectually prevents the development of piroplasmosis by destroying the parasites at the onset of infection.

2. Trypanblau given by the mouth is ineffective, since it exerts no apparent influence either upon the parasite or upon the course of the disease.

3. Tryparosan, when injected subcutaneously or when given by the mouth, has no effect upon the parasite and is ineffective as a remedy against piroplasmosis in the dog.

It remains to be determined how long Trypanblau (and Trypanrot ?) are capable of protecting animals exposed to infection.

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THE DRUG TREATMENT OF PIROPLASMOSIS IN CATTLE.

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(7 charts.)

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Introduction.

In a previous paper[1] we described experiments upon the curative and preventive treatment of canine piroplasmosis by means of trypanblau and trypanrot and briefly stated that trypanblau exerts similar

[1] Nuttall, G. H. F., and Hadwen, S., (1909). "The successful Drug treatment of canine piroplasmosis, together with observations upon the effect of drugs on *Piroplasma canis.*" *Parasitology*, II. pp. 156—191, 1 text-figure.

effects upon the bovine parasite to those observed in the case of *Piroplasma canis.* The object of this paper is to describe in detail our experiments upon the curative treatment of bovine piroplasmosis (Redwater or Texas Fever) by means of trypanblau.

Our experiments on Redwater were rendered possible through the help of the Colonial Office and of the Board of Agriculture and Fisheries. We are much indebted to H. J. Read, Esq., C.M.G., of the Colonial Office, for the kind interest he has taken in our work, and we take this occasion to thank Messrs Stewart Stockman, M.R.C.V.S. (Chief Veterinary Officer of the Board), James R. Jackson, M.R.C.V.S., and W. G. Wragg, M.R.C.V.S., for the very friendly help they gave us during the prosecution of our experiments in the Laboratory of the Board of Agriculture and Fisheries at Alperton, Wembley, Middlesex.

History of the strain of Piroplasma bovis used in these experiments.

We are indebted to Mr J. R. Jackson for the following particulars regarding the strain of South African *Piroplasma bovis* used for the inoculation of the cattle upon which we experimented:

No. of passages through animals

1. *Initial Case.* On 9. VII. 1904 a calf, 3 months old, was infested with *Boophilus decoloratus* larvae obtained from South Africa. The calf did not develop symptoms, and no *P. bovis* were found in its blood. (Sir John MacFadyean's case.)
2. *Yearling Bull* 9 was inoculated 13. IV. 1905 with 5 c.c. of defibrinated blood taken from the initial case. The bull developed no symptoms, but a few *P. bovis* were detected in its blood on 22. IV. 1905.
3. *Heifer* 5 was inoculated on 2. XII. 1905 with 5 c.c. of undefibrinated blood taken from the jugular vein of Bull 9. The heifer developed fever and *P. bovis* were detected in its blood on 11. XII. 1905. The heifer suffered from severe piroplasmosis but made a slow and steady recovery.
4. *Heifer* 108 was inoculated 5. IX. 1907 with 50 c.c. of defibrinated blood from Heifer 5. The animal developed fever and *P. bovis* were detected in its blood on 13. IX. 1907; the case was a severe one but recovery was rapid.

With a view to assuring ourselves of the virulence of the strain maintained in Heifer 108 since September, 1907, two cows were inoculated by Mr Stockman with its blood as follows:

5. *Cow* X was inoculated 22. V. 1909 with 30 c.c. of defibrinated blood from Heifer 108, but having failed to react (see Protocol, p. 241) the cow was reinoculated after 13 days with 200 c.c. of defibrinated blood from Heifer 108. The cow showed parasites on the 16th day after the first inoculation.

No. of passages through animals

Cow 5 (which received treatment) was inoculated 3. vi. 1909 with 200 c.c. of defibrinated blood from Heifer 108, but having failed to react by the 13th day the cow was reinoculated on 15. vi. 1909 with 30 c.c. of jugular blood taken 2 hours after death from our Control Cow I.

6. *All of our remaining cows* (Controls I—IV, Treated Cows 1—4), 8 in all, were each of them inoculated with 30 c.c. of defibrinated blood from Cow X on 7. vi. 1909. As will be seen by reference to their protocols (pp. 244, 252 *et seq.*) all of these animals developed piroplasmosis.

As will be seen by reference to the foregoing record the history of the strain starts with (1) *The initial case* which was induced by means of infected ticks. This and the succeeding case, the first due to inoculation, ran a very mild course. The cases induced by subsequent inoculations (Passages 3 and 4, etc.) were severer.

The Cattle used for our Experiments.

The animals used in our experiments were purchased in the open market. In view of the limited means at our disposal, the cattle were of inferior quality. The majority were shorthorns,—two were Jerseys. Cows IV (Control) and 4 (Treated) were in poor condition at the start. Excluding Cow X, with whose blood eight of the experimental animals were inoculated, we had nine animals at our disposal for the purpose of experiment. Of these animals four served as Controls and five were subjected to treatment.

Methods of Investigation.

The cows were in all cases inoculated subcutaneously with blood obtained from the jugular vein of animals infected with *P. bovis.* In most instances the blood had been defibrinated prior to injection. The Trypanblau was prepared in saturated watery solution (cold) and injected in quantities of 130 to 200 c.c. Four of the treated cows received the drug intravenously, whilst the fifth cow was treated subcutaneously.

The rest of our technique was precisely the same as that adopted in our experiments on dogs. Consequently we refer the reader to our previous paper (particularly to p. 161) where we describe our method of enumerating the different forms of intracorpuscular parasites as recorded in the protocols.

In the blood counts which accompany all our protocols the percentage of infected corpuscles was usually determined by counting 500

consecutive corpuscles *along the middle* of the blood film, and noting the number which harboured parasites. Where there were fewer than about two infected r.b.c. per 500 counted, we estimated the percentage of infected corpuscles by the examination of successive "fields" each containing approximately the same number of corpuscles. The percentage of each class of parasite was usually determined by counting 100 consecutive infected corpuscles and classifying them according to the types of parasites which they contained. At times we counted 200 infected corpuscles for this purpose; at other times, where few parasites were present, we had to roughly base our percentage on smaller numbers (as stated in the protocols).

Observations on Untreated Cattle.

The strain of *P. bovis* we used was not nearly so virulent as that of *P. canis*. Whereas all of our Control dogs died from piroplasmosis only one out of four Control cows died of redwater. As will be seen (p. 240), only two of the untreated cows had haemoglobinuria. In both cases the urine was very heavily charged with haemoglobin. One of these cows died of piroplasmosis.

Notes regarding Piroplasma bovis.

Owing to certain differences in the morphology of *P. bovis* as compared to *P. canis* we have contented ourselves with a simpler classification of the types of *P. bovis* encountered in corpuscles. We omit to classify the single intracorpuscular pyriforms (P) separately, because many oblong, ovoid or pyriform *P. bovis* occur which represent growing forms and not parasites which have quite recently invaded the corpuscles. This is in marked contrast to what is seen in *P. canis*, where the pyriforms soon become rounded after entering corpuscles. We therefore, in the case of *P. bovis*, include all single rounded, irregular, ovoid or pyriform parasites under the sign (O). As in *P. canis*, the (O) and double pyriform parasites (PP) predominate in respect to numbers. On the other hand dividing forms (D) occur more frequently and in greater numbers than in *P. canis*, which indicates that the process of division lasts longer in *P. bovis* than in the dog parasite.

Observations made by one of us (G. H. F. N.) on *P. bovis* in fresh blood maintained at 37° C. certainly indicate that the bovine parasite

is less active than the canine both as to movement and rate of multiplication.

The signs, used in our protocols, have the following signification:—

The sign	stands for a corpuscle containing
(O)	1 rounded, ovoid, pyriform, or irregular parasite.
(PP)	2 pyriform parasites.
(D)	1 dividing form.

The brackets ( ) represent the corpuscle which contains the parasites.

The abbreviation *r.b.c.* stands for red blood-corpuscle.

The sign + in the protocols denotes that the number of infected r.b.c. etc. was too small to determine the percentage.

The percentage of infected corpuscles in our untreated cattle never rose to the height observed in dogs. On the other hand, whilst the highest percentage of (D) encountered in dogs was 5—6 %, these forms at times occurred in 16 % of the parasitized corpuscles in cattle. The following table summarizes the observations above-mentioned:

Cow No.	The maximum % of infected r.b.c. observed	The maximum % of (D) forms observed	Haemoglobinuria + present 0 absent
Cow X	0·8	4	0
Control I	5·0	13	+ (died)
,, II	0·06	8	0
,, III	0·2	16	0
,, IV	4·6	14	+

That the percentage of infected corpuscles may occasionally be large is shown by the counts we made on blood films from Heifer 182 (see p. 243). In this case 58·2 % of the corpuscles contained parasites on the day it died. This animal had haemoglobinuria.

Owing to the fact that we have included (P) forms under class (O) the percentage of the latter is usually in excess of the (PP) forms. The percentage of (PP) forms may, however, vary in individual cows at different times, as is shown in the following table:

	Percentage of (PP) varied between
Cow X	52 and 36
Control I	48 ,, 33 (died)
,, II	43 ,, 29
,, III	38 ,, 16
,, IV	48 ,, 31
Heifer 182	36 ,, 19·5
And before treatment in	
Cow 1	42 ,, 33
,, 2	52 ,, 41
,, 3	53 ,, 27
,, 4	37 ,, 33
,, 5	43 ,, 30

In all the untreated cases of redwater which we have studied the (PP) forms were seen, as in dogs, to persist in the blood up to the death of the animals (see Control I, p. 244; Heifer 182, p. 243), or else they disappeared simultaneously with the (O) forms when the parasites vanished and recovery took place (see Cow X, p. 242; Controls II—IV, pp. 245 *et seq.*).

In our untreated cows the parasites were observable microscopically in the blood for four to six days, after which they could not be detected for two to 11 days:

	Parasites found microscopically in blood-films during	After which they disappeared for
Cow X	4 days	11 days
Control I (died)	4 ,,	—
,, II	5 ,,	8 ,,
,, III	6 ,,	2 ,,
,, IV	5 ,,	8 ,,

In all of the four cows which recovered naturally the parasites reappeared in very small numbers (only found at the edge or end of stained blood-films) on one or several days as will be seen by reference to the protocols.

Record of Cow X.

	Day	Temp. °F. morning & evening	Cow in poor condition.
(22. v. 09.)	1	—	*Inoculated* with 30 c.c. of defibrinated blood from Heifer 108.
	2	103·0	
		101·8	
	3	102·0	
		104·0	
	4	101·6	Blood examined twice daily but no parasites found.
		101·2	
	5	101·0	Ditto.
		101·8	
	6	102·4	Ditto.
		102·4	
	7	102·4	Ditto.
		104·4	
	8	102·2	Ditto.
		104·2	
	9	101·0	Ditto.
		104·0	
	10	103·2	Ditto.
		105·2	
	11	103·8	Ditto.
		104·0	
	12	102·8	Ditto.
		103·8	

Day	Temp. °F. morning & evening		% of infected r.b.c.	% O	% PP	% D
13	103·0 103·0	Ditto. *Reinoculated* with 200 c.c. of blood from Heifer 108 at 4 p.m.				
14	102·2 101·2	Blood examined twice daily but no parasites found.				
15	103·0 103·0	Ditto.				
16	105·0 106·0	*Parasites found*, very few at film-end in leucocyte clumps, only 25 infected r.b.c. found in the morning, a few more in the afternoon	+	48	52	?
17	105·2 105·4	A few parasites at film-edge. (Blood taken for inoculation into 8 cows) ...	+	53	45·5	1·5
18	105·2	More parasites, in 500 r.b.c. counted there were, at 11 a.m.	·8	57·5	39·5	3
	105·2	Fewer parasites, in 500 r.b.c. counted there were, at 4.45 p.m.	·2	47	49	4
19	103·2	Fewer parasites, in over 1000 r.b.c. counted there were, at 9 a.m.	·1	64	36	
	104·0	Fewer parasites, only found at film-edge, at 3.45 p.m.	+	+	+	
20	101·2 104·8	*No parasites found* morning or evening ...	0			
21	102·4 104·2	*No parasites found*, examined once daily ...	0			
22	102·0 103·6	Ditto	0			
23	102·8 105·0	Ditto	0			
24	102·4 105·0	Ditto. Cow feeding	0			
25	102·6 105·0	*No parasites found*, examined once daily ...	0			
26	102·0 105·2	Ditto. Cow not feeding	0			
27	102·8 105·6	*No parasites found*, examined once daily. Urine normal. Haematocrit reading = 56 % r.b.c.	0			
28	101·8 103·8	*No parasites found*, examined once daily ..	0			
29	101·8 104·8	Ditto	0			
30	101·0 104·0	Ditto	0			
31	102·2 103·6	One infected r.b.c. found, 1 (PP) ...	+			
32	103·0 102·4	*No parasites found*, examined once daily. Haematocrit reading = 57 % r.b.c. ...	0			

	Day	Temp. °F. morning & evening		% of infected r.b.c.
	33	102·6	*No parasites found*, examined once daily	0
		105·0		
	34	104·6	1 infected r.b.c. found, 1 (O)	+
		105·0		
	35	104·2	*No parasites*. Cow much emaciated ...	0
		101·2		
	36	104·0		
		105·2		
	37	102·8		
		104·8		
	38	104·0		
		104·8		
(29. VI. 09)	39	104·0	Observations ceased.	

Record of Heifer 182.

Although Heifer 182 does not belong to the series of cows which served for our experiments, we have decided to include its record because of its interest. The animal died from piroplasmosis, a very high percentage of infected corpuscles (58·2 %) being found on the day when it died. We are indebted to Mr J. R. Jackson for the clinical notes of the case and our thanks are due to Mr W. G. Wragg for allowing us to examine a series of blood-films taken from this animal.

In the following table we in each instance counted 500 corpuscles to determine the percentage of infected r.b.c. In the blood-film taken on the 11th day we counted 100 parasitized r.b.c., in the other films we counted 200 parasitized r.b.c. to determine the percentages of the different forms of parasites contained in the corpuscles.

	Day	Temp. °F. morning & evening		% of infected r.b.c.	% (O)	% (PP)	% (D)
(13. II. 09)	1	—	*Inoculated* with defibrinated blood of Heifer 108 (see p. 237).				
	9	103·0					
	10	104·0	*Parasites appeared*, 1 (PP) observed ...	+	·	+	·
		104·0					
	11	103·0	Parasites increasing	0·2	64	36	?
		104·0					
	12	104·0	Parasites increasing	2·0	68	24	8
		106·0					
	13	106·0	Cow not feeding. *Haemoglobinuria* ...	7·4	70	22·5	7·5
		105·0					
(26. II. 09)	14	107·0	Heavy infection. Heifer died at 12.30 p.m.	58·2	75	19·5	5·5
		106·4					

The autopsy made on the day after death revealed the typical appearances of animals which have died of Redwater.

Records of Control Cows I to IV.

Control Cow I.

	Day	Temp.°F.		% of infected r.b.c.	% O	% PP	% D
(7. VI. 09)	1	—	*Inoculated* with 30 c.c. of virulent blood (from Cow X).				
	2	102·0	a.m.				
		101·6	p.m.				
	3	101·4	a.m.				
		101·6	p.m.				
	4	101·2	a.m.				
		101·8	p.m.				
	5	101·0	a.m.				
		102·6	p.m.				
	6	105·2	9 a.m.				
			11.30 a.m. *Parasites found*	·05	55	37	8
		106·2	5.30 p.m.	0·6	59	36	5
	7	107·4	9.30 a.m. *Haemoglobinuria*, urine very dark	4·0	64	33	3
		108·4	4.45 p.m. Cow very ill	2·2	54	33	13
			7.15 p.m.	5·0	49	45	6
		106·2	9 p.m.				
	8	103·0	9.30 a.m.				
			11 a.m.	3·2	53	45	2
			2.40 p.m. Haemoglobinuria more marked than on previous day.				
		101·6	4 p.m.				
			5.30 p.m.	1·2	49	48	3
			7.15 p.m. Cow very feeble, breathing rapid.				
			8 p.m.	1·0	55	41	4
(15. VI. 09)	9	99·4	10 a.m. Very feeble and ill. Haemoglobinuria.				
			11 a.m.	0·1	61	37	2
			1.50 p.m. Haemoglobinuria slightly less marked. *Cow died.*				

Autopsy:—The animal showed typical appearances, spleen engorged, friable, much enlarged (weight 7—8 lbs.); liver jaundiced; kidneys with haemorrhagic infarcts at cortex; heart with petechiae dotting the pericardium and muscle; lungs normal; brain anaemic; blood very watery. Microscopic examination of smears made from the jugular blood and various organs two hours after death revealed very few parasites; only 5 infected blood corpuscles were found in a spleen smear [(PP) and (O)] and fewer still were found in smears from the liver, brain, and bone-marrow; none were found in smears from the kidneys, lungs, and heart-muscle.

Control Cow II.

	Day	Temp. °F.		% of infected r.b.c.	% O	% PP	% D
(7. VI. 09)	1	—	*Inoculated* with 30 c.c. of virulent blood (from Cow X).				
	2	101·4	a.m.				
		102·0	p.m.				
	3	101·6	a.m.				
		101·0	p.m.				
	4	101·6	a.m.				
		101·4	p.m.				
	5	102·0	a.m.				
		102·0	p.m.				
	6	102·0	a.m.				
		106·0	5.30 p.m. *Parasites found*, very few at film-end, only 25 infected r.b.c. found ...	+	64	32	4
	7	102·4	9.45 a.m.	·06	63	34	3
		103·4	4.45 p.m.	·02	67	29	4
	8	102·2	9.30 a.m.				
			11 a.m.	·01	49	43	8
		101·8	4 p.m.				
	9	101·6	10 a.m.				
	10	101·6	— a.m. Cow appears well. Very few parasites (50 counted)	+	64	32	4
		102·0	— p.m.				
	11	101·8	9.30 a.m. Blood examined at 11 a.m.: *No parasites* found	0			
		102·2	6.30 p.m.				
	12	102·0	9.30 a.m. Blood examined at 11 a.m.: *No parasites* found	0			
		101·4	4 p.m. Blood examined at 5.30 p.m. with haematocrit = 102 % r.b.c.				
	13–18	Normal	No parasites found. Haematocrit reading on 17th day = 90 % r.b.c.	0			
	19	102·0	10 a.m. Only 1 infected r.b.c. found in film: 1 (O)	+			
		101·8	5 p.m.				
	20	102·0	— a.m.				
		101·2	— p.m. No parasites found	0			
	21–22	Normal.					
(28. VI. 09)	23	,,	No parasites	0			
	24	,,	a.m. Observations ceased.				

Control Cow III.

Estimated weight 80 st.

	Day	Temp. °F.		% of infected r.b.c.	% O	% PP	% D
(7. vi. 09)	1	—	*Inoculated* with 30 c.c. of virulent blood from Cow X.				
	2	102·0	a.m.				
		103·0	p.m.				
	3	105·0	a.m.				
		104·0	p.m.				
	4	104·8	a.m. *Parasites found:* only 6 infected r.b.c. found amongst leucocytes.				
		104·2	p.m.				
	5	106·2	9.30 a.m.	·03	59	38	3
		105·6	p.m.				
	6	103·4	9.30 a.m. Cow weak. Blood examined at 11.30 a.m.	0·2	74·5	23·5	2
		104·2	5.30 p.m.	·06	74	24	2
	7	103·2	9.50 a.m. Very few parasites at film-edge, only 25 counted	+	68	16	16
		105·0	4.45 p.m.	·05	68	28	4
	8	101·0	9.30 a.m. Cow chewing her cud, appears well. Blood examined at 11 a.m. and only 3 infected r.b.c.: 1 (O), 2 (PP) found at film-edge after long search ...	+			
		102·4	4 to 4.40 p.m. Urine normal; has not had haemoglobinuria.				
	9	102·0	9 a.m.				
			3.30. Cow feeding well, urine normal, 1 infected r.b.c. (O) found at film-edge ...	+			
		101·8	5 p.m.				
	10	103·0	9.30 a.m. No parasites	0			
		104·0	6.30 p.m.				
	11	102·0	9.30 a.m. No parasites found at 11 a.m., but many punctate r.b.c.	0			
			3.30 p.m. Urine normal. Haematocrit reading = 58 % r.b.c.				
		102·6	4 p.m.				
	12	101·0	9.30 a.m. 2 infected r.b.c. found, 2 (PP)	+			
		102·2	5.30 p.m.				
	13	102·6	9.30 a.m. 2 infected r.b.c. found, I (O), 1 (PPP)	+			
		101·4	4 p.m.				
	14	102·0	10 a.m. 1 infected r.b.c. found, 1 (O) ...	+			
		104·0	5 p.m.				
	15	102·0	10 a.m. Normal urine; no parasites.				
		103·6	4 p.m. At 5.45 haematocrit reading = 56 % r.b.c.	0			
	16	103·2	10 a.m. Normal urine; no parasites ...	0			
		102·8	5 p.m.				
	17	103·0	10 a.m. No parasites found	0			
		103·5	5 p.m.				
	18	102·2	10 a.m. 1 infected r.b.c. (O) found ...	+			
		102·6	5 p.m.				
	19	103·2	a.m. No parasites found	0			
		102·6	p.m.				
	20	101·6	a.m.				
		101·1	p.m.				
	21	102·0	a.m.				
		101·6	p.m.				
	22	102·8	a.m. No parasites found	0			
		104·0	p.m.				
(29. v. 09)	23	103·4	a.m. Observations ceased.				

Control Cow IV.

Estimated wt. 60 st. Cow in poor condition.

	Day	Temp. °F.		$^0/_0$ of infected r.b.c.	$^0/_0$ O	$^0/_0$ PP	$^0/_0$ D
(7. vi. 09)	1	—	*Inoculated* with 30 c.c. of virulent blood from Cow X.				
	2	101·0	a.m.				
		102·0	p.m.				
	3	101·2	a.m.				
		103·0	p m.				
	4	102·4	a.m.				
		104·0	p.m.				
	5	101·2	a.m.				
		102·2	p.m. *Parasites appeared* (3 p.m.). Very few: (PP) and (O) found	+			
	6	101·4	a.m. Few parasites, 8000 r.b.c. counted to obtain $^0/_0$ of infected r.b.c.	·01	62	34	4
		105·0	p.m. More parasites	0·1	47	39	14
	7	106·6	a.m. ,, ,, ...	0·8	48	48	4
		107·6	p.m. ,, ,, Many faint r.b.c. *Haemoglobinuria* very dark ...	4·6	51	39	10
	8	101·6	a.m. Fewer parasites	0·2	66	31	3
			2.40 p.m. Haemoglobinuria much more marked than on Day 7.				
		101·4	4 p.m. At 5.30 p.m. blood examined ...	·03	54	42	4
			7.15 p.m. cow feeding. At 8 p.m. blood examined	0·5	54	42	4
	9	102·0	10 a.m. At 11.10 a.m. very few infected r.b.c.	+	52	46	2
			2.30 p.m. Haemoglobinuria to same degree as before. Blood watery.				
			3.30 p.m. Cow feeding well.				
		102·0	5 p.m.				
	10	101·2	9.30 a.m. Not feeding.				
			11 a.m. Blood watery. Haemoglobinuria much less marked. No parasites ...	0			
		103·6	6.30 p.m.				
	11	101·0	9.30 a.m. Blood examined at 11 a.m., no parasites, punctate r.b.c.	0			
			3 p.m. Haemoglobinuria slight. Haematocrit reading = 18 $^0/_0$ r.b.c.				
		102·6	4 p.m.				
	12	101·0	9.30 a.m. Urine normal. No parasites ...	0			
		103·2	5.30 p.m.				
	13	102·6	a.m. No parasites	0			
		102·2	p.m.				
	14	101·6	a.m. ,, ,, ...	0			
		103·6	p.m.				
	15	102·2	a.m. ,, ,, ...	0			
		103·8	p.m. At 6 p.m. urine normal. Haematocrit reading = 29 $^0/_0$ r.b.c.				
	16	103·2	a.m. No parasites	0			
		102·4	p.m.				
	17	101·6	a.m. ,, ,, ...	0			
		102·2	p.m.				
	18	103·0	a.m. 2 infected r.b.c. found: 1 (PP) and 1 (O)	+			
		102·8	p.m.				
(25. vi. 09)	19	102·8	a.m.				
			3 p.m. 3 infected r.b.c. found: 2 (PP) and 1 (O)	+			

Cow killed: organs normal but for presence of Johne's disease.

Observations on Treated Cattle.

The beneficial effects of trypanblau treatment in cattle are not as striking as in dogs, because the disease in the cattle upon which we experimented was much less fatal than that in dogs. Nevertheless, the curative effect is quite evident when we observe the action of the drug upon the parasites and upon the symptoms of the disease and compare the data relating to the treated animals with those relating to the controls.

If we accept the presence of *Haemoglobinuria* as an indication of the severity of the disease, it follows that the cases chosen for treatment chanced to be severer, taken as a whole, than the group of cases chosen to serve as controls.

The Controls. Haemaglobinuria was observed in two out of the four Controls; in Control Cow I it followed 24 hours after the appearance of parasites, lasted three days, and persisted until death; in Control Cow IV haemoglobinuria followed 48 hours after the appearance of the parasites and persisted for five days. In both cases haemoglobinuria was severe. It is worthy of note that one of these two cows which had haemoglobinuria died from piroplasmosis.

The treated animals. All of the five treated animals had haemoglobinuria, but recovered. In four the haemoglobinuria appeared on the day the animal was treated, but before the injection of the drug. In one cow (No. 1) haemoglobinuria was first noted 24 hours after treatment. The haemoglobinuria was severe in Cows 3 and 4, marked in Cows 1 and 5, slight in Cow 2.

Haemoglobinuria was noted in		Haemoglobinuria lasted
Cow 1	48 hours after the parasites appeared	24 hours.
,, 2	42 ,, ,, ,, ,,	1 day.
,, 3	22 ,, ,, ,, ,,	5 days.
,, 4	when the parasites were first found	36 hours.
,, 5	49 hours after the parasites appeared	48 ,,

To repeat: Whereas one of the two controls which had haemoglobinuria died of piroplasmosis, all of the five treated animals recovered, although they had haemoglobinuria. In the untreated cows haemoglobinuria lasted in one case three days when the animal died, in the other case five days when the animal recovered. In only one treated cow (3) did the haemoglobinuria last five days, in the others it lasted only 12, 24, 36 and 48 hours respectively. The differences in this respect are shown graphically in the charts (pp. 259 *et seq.*).

The Effects of Trypanblau upon the Parasites.

The most striking proof of the efficacy of the drug is, however, afforded by the obvious effect it exerts upon the parasites. Exactly as in *P. canis*, the (D) forms are the first to disappear, then the (PP) forms, only the rounded or irregular forms (O) persisting for a time, whilst the percentage of infected r.b.c. falls rapidly. The effects of trypanblau are clearly set out in the following tables and the charts on pp. 259 *et seq.* Cow 5 received the drug subcutaneously, which accounts for the slower effect exerted upon the parasites.

Blood examination before treatment.

Cow	Maximum percentage of infected r.b.c.	Maximum percentage of (D) forms
1	0·8	8
2	1·0	8
3	1·0	11
4	0·6	3
5	1·5	6

N.B.—In none of the treated animals did the number of infected corpuscles exceed 1·5 %. In two out of four controls (see p. 240) no less than 4·6 % and 5 % of infected r.b.c. are recorded. In only one of the treated cows did the number of (D) forms attain 11 %. In three out of four controls, 13 %, 14 % and 16 % of (D) forms were recorded. These differences are attributable to the treatment.

Table showing the effect of the drug in reducing the percentage of infected corpuscles within $4\frac{1}{4}$ *to 8 hours after treatment and causing the disappearance of* (PP) *and* (D) *forms.*

Treated Cow No.	Showing effect of drug after stated time had elapsed	Percentage of infected r.b.c.		Percentage of (PP) forms		Percentage of (D) forms	
		At time when drug was given	After drug was given	At time when drug was given	After drug was given	At time when drug was given	After drug was given
1	$4\frac{1}{4}$ hours	0·8	·01	33	1	4	0
2	8 ,,	1·0	·03	41	1	8	0
3	$4\frac{3}{4}$,,	0·6	·06	44	2	5	0
4	$4\frac{1}{2}$,,	0·6	·03	33	3	2	0
5*	7 ,,	1·5	·02	33·5	5·5	4	1

* This case was treated by subcutaneous injection. The effect of the drug is obviously less rapid.

After the periods of time noted in the above table, the blood was repeatedly examined for parasites. The results of these examinations are summarized in the following table.

Table showing the time when the cows were treated after the parasites had first been found, the time which elapsed between the injection of the drug and the disappearance of the parasites, and the time when the parasites reappeared after their first disappearance.

Treated Cow No.	Time in days when cow was treated after parasites appeared	Time it took the parasites to disappear after drug was injected	Time when parasites reappeared
1	1 day	– 24 hours*	Blood negative for 18 days.
2	2 days	– 45 ,,	,, ,, ,, 16 ,,
3	1 day	– 24 ,,	,, ,, ,, 6 ,, Very few parasites found later, none on some days.
4	Same day	9 ,,	Blood negative for 5 days, 1 parasite found 6th day, none later.
5	2 days	– 45 ,,	Blood negative for 6 days when observation ceased.

* The – signs before the numbers denote that the parasites may have disappeared some hours earlier than is recorded.

Note:—As will be seen by reference to p. 241 the parasites were found microscopically in the blood of the Control Cows during 4 to 6 days. The parasites reappeared after 2 days in one case, after 8 days in two cases, after 11 days in one case.

Judging from the facts above recorded, it appears certain that some of these cows would have died but for the treatment they received.

Note regarding degenerative changes in the parasites.

The single parasites contained in infected corpuscles showed evidence of degeneration, as described in *P. canis,* in consequence of the treatment. When these single parasites were grouped according to their size into "large" and "small" we observed the following changes in their relative numbers as the result of treatment. (Examination of stained films from Cow 1):

At 12.30 p.m., just before treatment there were 66 % large and 34 % small parasites.
,, 2.45 p.m., after ,, ,, 35 % ,, 65 % ,, ,,
,, 4.45 p.m., ,, ,, ,, 36 % ,, 64 % ,, ,,

After treatment, the large intracorpuscular parasites stained *faintly* in contrast to the small parasites, which stain intensely. At the same time a number of conjoined pairs of free pyriform parasites were observable which stained faintly, and single degenerated pyriforms could be occasionally encountered.

Some Haematocrit readings in untreated and treated Cows.

The protocols include some examinations of the blood by means of the haematocrit. The observations, whilst suggestive, are too few in number to permit of any definite conclusions. We regret that time did not permit of our making systematic examinations of the blood by means of the haematocrit, haemocytometer, etc. Such observations should be made in conjunction with future experiments of this character. The haematocrit readings, made only twice for each cow, gave the following figures, which appear to speak in favour of the treatment, since only rises are recorded in the °/₀ of r.b.c. in the treated animals, whereas in two out of three control animals the °/₀ of r.b.c. appears to fall.

Control Cow No.	Days after inoculation when blood was examined	Percentage r.b.c. on the two days	Indicating
II	12, 17	102, 90	A fall.
III	11, 15	58, 56	A slight fall.
IV	11, 15	18, 29	A rise.
Treated Cow No.	...	...	
1	11, 16	100, 116	A rise.
2	11, 16	72, 84	,,
3	11, 15	32, 37	A slight rise.
4	11, 16	68, 71	,, ,,

Records of Treated Cows 1 to 5.

Treated Cow 1.

Estimated weight 110 st.

	Day	Temp.°F.		% of infected r.b.c.	% O	% PP	% D
(7. VI. 09)	1	—	*Inoculated* with 30 c.c. of virulent blood from Cow X.				
	2—3	Normal.					
	4	105·0	9 a.m. Blood examined at 11 am. *Parasites found*	·05	54	41	5
			3 p.m.	·1	53	42	5
		105·2	5 p.m.				
	5	107·2	9.30 a.m. Cow looks ill	·4	55	37	8
			12.30 p.m. *Treatment:* 200 c.c. sat. sol. Trypanblau intravenously	·8	63	33	4
			2.45 p.m.	·2	85	15	·
		110 (!)	4.30 p.m.				
		108·8	4.45 p.m.	·01	99	1	·
		107·8	5.45 p.m.				
		103·0	7 p.m. only 5 infected r.b.c. found at film-end; parasites degenerated ...	+			
		101·8	9 p.m. only 1 infected r.b.c. found at film-end; parasites degenerated ...	+			
			The cow appeared better at 7 p.m. and at 9 p.m. began to feed.				
	6	Normal.	Cow lively and feeding. *No parasites* ...	0			
			11 a.m. *Haemoglobinuria* marked.				
	7	,,	Cow lively. No parasites. Running from eyes	0			
			7 p.m. Urine almost normal.				
	8—10	,,	Cow looking well. Urine normal. No parasites	0			
	11	,,	Ditto. Haematocrit reading = 100 % r.b.c.	0			
	12—15	,,	Ditto. No parasites	0			
	16	,,	Ditto. Haematocrit reading = 116 % r.b.c.	0			
	17—19	,,	Cow lively and well. No parasites ...	0			
	20—21	,,					
	22	,,	No parasites	0			
(29. VI. 09)	23	,,	Observations ceased.				

Treated Cow 2.

Estimated weight 100 st.

	Day	Temp. °F.		% of infected r.b.c.	% O	% PP	% D
(7. VI. 09)	1	—	*Inoculated* with 30 c.c. of virulent blood from Cow X.				
	2—3	Normal.					
	4	103·0	9 a.m.				
			3 p.m. *Parasites appeared*, very few at edge of film in clumps of leucocytes.				
		101·0	5 p.m.				
	5	102·8	9 a.m.	·05	43	52	5
		103·0	5 p.m.				
	6	105·4	9 a.m. *Haemoglobinuria* (slight) at 10.30 a.m.				
			11 a.m.	0·8	53	44	3
			1 p.m. *Treatment:* 130 c.c. sat. sol. Trypanblau injected intravenously ...	1·0	51	41	8
			2.30 p.m.	0·4	48	52	·
			4.10 p.m. (14 PP were free, but connected at their tips, they stained faintly and appeared degenerated)	·01	69	29	2
		107·8	5.30 p.m. (8 PP encountered free as at 4.10 p.m.)	·06	88	11	1
			7 p.m. (degenerating PP forms encountered)	·04	98	1	1
		104·8	9 p.m. (ditto)	·03	99	1	·
	7	Normal	6.30 a.m. Cow looks lively.				
		,,	9.30 a.m. Only 1 (O) infected r.b.c. found, the parasite degenerated	+			
		,,	5 and 9 p.m.				
	8	,,	a.m. and p.m. *No parasites*, urine normal, cow looking well	0			
	9—10	,,	Ditto	0			
	11	,,	Ditto. Haematocrit reading = 72 % r.b.c.	0			
	12—15	,,	Ditto	0			
	16	,,	Ditto. Haematocrit reading = 84 % r.b.c.	0			
	17—19	,,	Ditto	0			
	20—21	,,					
	22	,,	No parasites	0			
(29. VI. 09)	23	104·0	a.m. Observations ceased.				

Treated Cow 3.

Estimated weight 104 st.

	Day	Temp. °F.		% of infected r.b.c.	% O	% PP	% D
7. vi. 09)	1	—	*Inoculated* with 30 c.c. of virulent blood from Cow X.				
	2—4	Normal	(morning and evening temperatures).				
	5	102·0	a.m.				
		103·0	p.m.				
	6	105·0	a.m. At 11 a.m. *Parasites found* ...	·06	62	27	11
		104·0	p.m. At 5.30 p.m. ,, ,, ...	·03	54	35	11
	7	107·0	9 a.m. *Haemoglobinuria*, very dark.				
			9.55 a.m.	1	45	53	2
			12 midday. *Treatment:* 150 c.c. sat. sol. Trypanblau	0·6	51	44	5
			2.30 p.m.	0·1	67	32	1
			4.30 p.m. Haemoglobinuria, very dark, redder than before.				
			4.45 p.m.	·06	98	2	·
		107·2	5 p.m.				
			7.15 p.m. Only 4 infected r.b.c. found: 3 (O), 1 (PP), parasites degenerated ...	+			
		102·2	9 p.m. Cow feeding. Only 4 infected r.b.c. found: 4 (O) all degenerated ...	+			
	8	101·0	6.10 a.m. Haemoglobinuria, still dark but less marked.				
			10.30 a.m. Haemoglobinuria, less than at 6.10 a.m.				
			12.15 a.m. No parasites	0			
			2.20 p.m. and 3.45 p.m. Haemoglobinuria less than at 10.30 a.m.				
		101·4	4 p.m.				
	9	101·4	10 a.m. At 11 a.m. Haemoglobinuria again very dark.				
			2.30 p.m. Cow feeding well. Blood watery.				
		102·2	4 p.m.				
	10	101·4	9.30 a.m. 11 a.m. blood watery, no parasites found	0			
			12 midday. Haemoglobinuria less marked.				
		102·2	6.30 p.m. Urine smoky.				

	Day	Temp. °F.		% of infected r.b.c.
	11	101·2	9.30 a.m. Feeding and looking better than day before.	
			11 a.m. No parasites, punctate r.b.c., some nucleated r.b.c.	0
			3 p.m. Urine smoky. Haematocrit reading = 32 % r.b.c.	
		103·0	4 p.m.	
	12	101·4	9.30 a.m. Urine normal. No parasites...	0
		101·0	5.30 p.m.	
	13	102·8	9.30 a.m. ,, ,, ,,	0
		103·0	4 p.m.	
	14	101·6	10 a.m. ,, ,, ,,	0
		102·0	5 p.m.	
	15	102·2	10 a.m. Urine normal. 2 infected r.b.c. found: 1 (PP), 1 (O)	+
		102·0	5.45 p.m. Haematocrit reading = 37 % r.b.c.	
	16	101·6	10 a.m. No parasites	0
		102·0	4 p.m.	
	17	101·2	10 a.m. 1 infected r.b.c. found: 1 (O) ...	+
		102·0	4 p.m.	
	18	102·0	10 a.m. No parasites	0
		102·0	4 p.m.	
	19	102·4	a.m. No parasites found	0
		101·0	p.m.	
	20	101·0	a.m.	
		101·8	p.m.	
	21	101·6	a.m.	
		101·8	p.m.	
	22	103·0	a.m. 3 infected r.b.c. found: 1 (PP), 2 (O)	+
		102·0	p.m.	
(29. vi. 09)	23	102·2	a.m. Observations ceased.	

Treated Cow 4.

In poor condition, estimated weight 104 st.

	Day	Temp. °F.		°/₀ of infected r.b.c.	°/₀ O	°/₀ PP	°/₀ D
(7. vi. 09)	1	—	*Inoculated* with 30 c.c. of virulent blood from Cow X.				
	2—6	Normal	(morning and evening temperature).				
	7	105·6	9.30 a.m. *Haemoglobinuria.* At 9.55 a.m. *Parasites found*	0·1	60	37	3
			12.10 p.m. *Treatment:* 150 c.c. sat. sol. Trypanblau	0·6	65	33	2
			2.30 p.m.	0·8	85	14	1
			4 p.m. Haemoglobinuria slightly increased.				
		107·0	4.45 p.m.	·03	97	3	·
			7.10 p.m. Only 3 infected r.b.c. found at film-end: 3 (O)	+			
		102·4	9 p.m. Cow looking better. Haemoglobinuria very dark. No parasites ...	0			
	8	101·2	6.10 a.m.				
		100·6	9.30 a.m. Chewing her cud. At 11.15 a.m. urine almost normal.				
			12.15 p.m. No parasites	0			
		102·2	4 p.m.				
	9	101·4	10 a.m. At 11.30 a.m. urine normal.				
		102·0	4 p.m. At 3.30 p.m. blood watery, no parasites	0			
	10	101·4	9.30 a.m. Cow looking well.				
			11 a.m. Urine normal, blood watery, no parasites	0			
		102·0	6.30 p.m.				
	11	101·0	9.30 a.m. At 11 a.m. no parasites found	0			
		102·0	4 p.m. Urine normal. At 5 p.m. Haematocrit reading = 68 °/₀ r.b.c.				
	12—14	Normal	(morning and evening temperature). No parasites	0			
	15	102·0	10 a.m. 1 infected r.b.c. found: 1 (PP) ...	+			
		102·2	4 p.m.				
	16	102·2	10 a.m. No parasites. Haematocrit reading at 2.30 p.m. = 71 °/₀ r.b.c. ...	0			
		102·2	4 p.m.				
	17—21	Normal	(morning and evening temperature). No parasites	0			
	22	,,	No parasites	0			
(29. vi. 09)	23	,,	a.m. Cow killed.				

Autopsy:—Blue colour distinctly noticeable in the subcutaneous tissues. No parasites found in smears from spleen, kidney, and liver. No Redwater lesions observed, the animal had Johne's disease.

Treated Cow 5.

Jersey. Estimated weight 80 st.

	Day	Temp.°F.		% of infected r.b.c.	% O	% PP	% D
(3. vi. 09)	1	102·0	*Inoculated*, 3.45 p.m., with 200 c.c. of defibrinated blood from Heifer 108.				
	2—12	Normal.	Animal appears well.				
	13	,,	*Reinoculated*, 3.30 p.m., with 30 c.c. of defibrinated blood taken from jugular vein of Cow 1 (Control), 2 hours after death.				
	14—17	,,	No parasites found to date.				
	18	104·0	10 a.m. *Parasites appeared*, few at film-edge	+	51	43	6
		104·4	5 p.m.				
	19	106·0	10 a.m. Blood examined at 11 a.m. ...	·01	57	40	3
		105·8	4 p.m. ,, ,, 5.30 p.m. ...	...	68	30	2
	20	105·2	9 a.m. ,, ,, 11 a.m. ...	·4	50	44	6
			11.25 a.m. *Haemoglobinuria.*				
			1 p.m. *Treatment:* 180 c.c. of sat. sol. Trypanblau subcutaneously ...	1·5	62·5	33·5	4
			2.30 p.m. Blood examination revealed ...	1·3	62·5	35	2·5
			3.45 p.m. Haemoglobinuria darker.				
			5 p.m. Blood examination revealed ...	0·1	—	—	—
			7 p.m. ,, ,, ,, ...	·05	97	2·5	·5
			8 p.m. ,, ,, ,, ...	·02	93·5	5·5	1
	21	Normal	11 a.m. Only 6 infected r.b.c. found: 6 (O) Haemoglobinuria less marked.	+	+		
	22	,,	Urine smoky. *No parasites*	0			
	23	,,	Urine normal ,, ,, ...	0			
	24—26	,,	No parasites	0			
(29. vi. 09)	27	,,	Observation ceased.				

Charts relating to the Cows which had Haemoglobinuria.

The accompanying charts relate to two cows (II and IV) which served as Controls, and to the five cows (1 to 5) which were treated. The object of the charts is to show graphically the chief facts recorded in the protocols: the sudden onset of fever; the appearance of parasites in the blood and their increase in numbers; the period when haemoglobinuria set in; the effect of treatment upon the foregoing symptoms and upon the parasites.

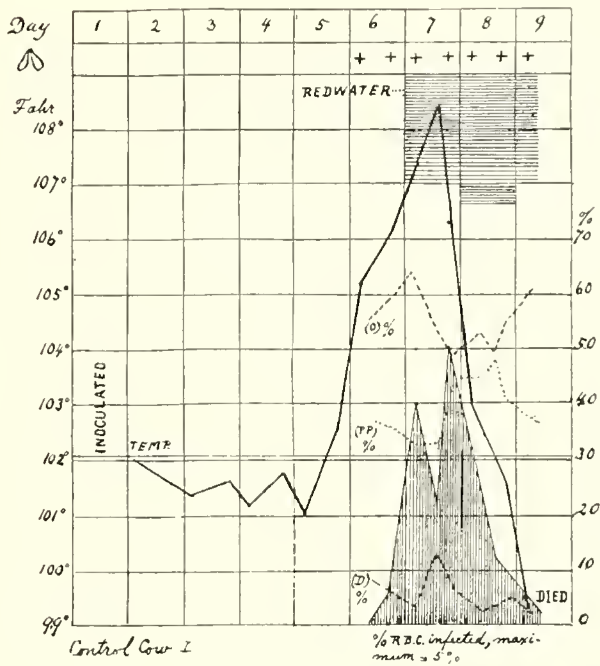
The numbers running along the top of the chart indicate the days on which the animal was observed, the inoculation being recorded on the first day, or subsequently when the animal received two inoculations. Beneath the day numbers the presence of parasites in the blood, irrespective of their number, is indicated by + signs. The average

normal temperature of 102° Fahr. is indicated by a double line in the chart. The rise and fall in the percentage of infected corpuscles is indicated below by vertical shading. Owing to the small number of parasites which was present in some cases the percentage of infection is not always drawn on the same scale in the different charts, but the shaded portion is drawn to scale for each chart, the maximum percentage of infected r.b.c. being stated below.

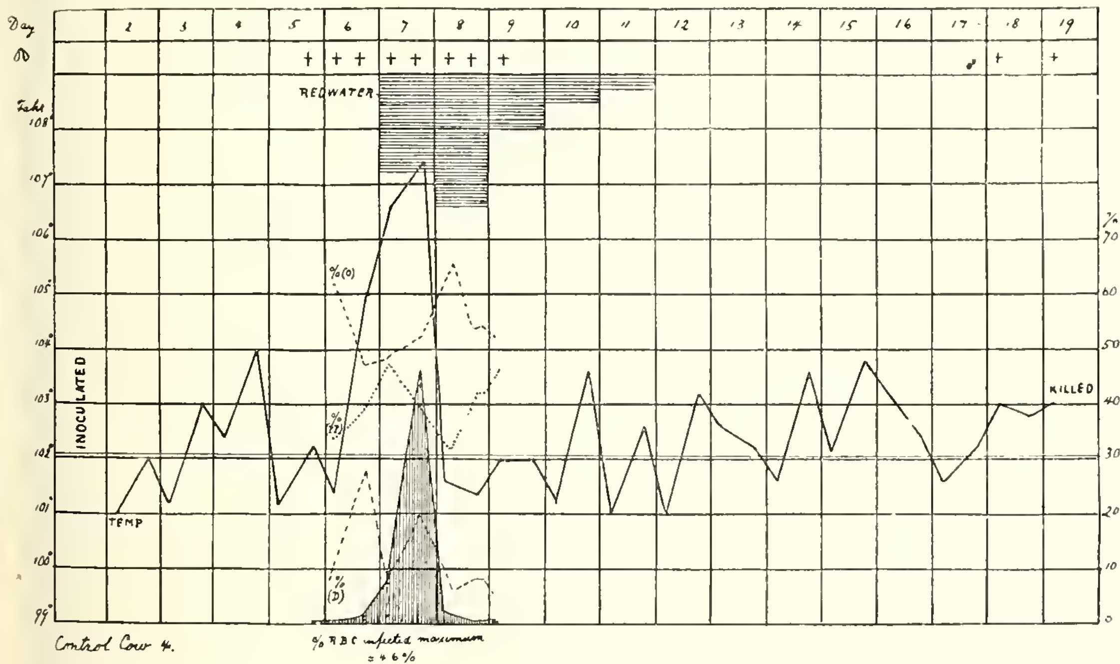
The variation in the relative percentages of the different types of parasites is given in accordance with the scale to the right of the chart. The percentage of rounded, ovoid or pyriform parasites (O) is the highest throughout and is indicated by a broken line. The percentages of double pyriform parasites (PP) and of dividing forms (D) are seen lower down on the chart.

The haemoglobinuria is indicated by the horizontal shading above; its intensity is represented by the amount of shaded area. Our method of determining the degree of haemoglobinuria was a rough one, but it sufficed for our purposes. It consisted in dipping strips of white filter paper into the samples of urine immediately after they were collected and allowing the strips to dry whilst suspended from a ledge. On each strip was noted the number of the cow and the time when the urine was collected. By spreading out the dried strips in a line the relative intensity of the colour of the paper was noted and the charts were shaded accordingly.

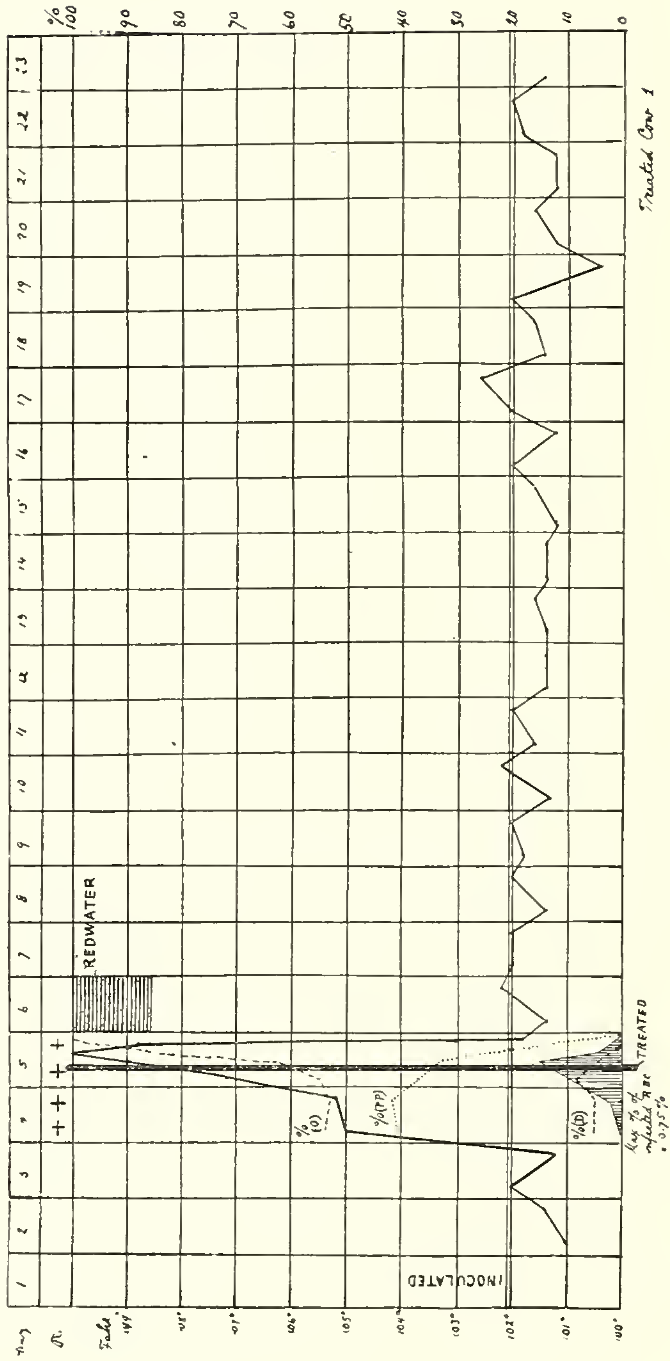
The time when the animals (1 to 5) were treated is indicated by a vertical black line. When we compare the charts relating to Cow I and II (untreated) with the charts relating to the treated animals, it will be noticed (*a*) that the percentage of infected red blood corpuscles was greatest in the untreated animals; (*b*) that the parasites persisted longer in the blood of the untreated animals (note the crosses above); (*c*) that the haemoglobinuria was not as intense in four out of the five treated cows as compared to the untreated animals; (*d*) that the percentage of infected corpuscles in the treated animals is seen to fall promptly after the injection of the drug; (*e*) that the dividing (D) and pyriform parasites (PP) disappear and are replaced by rounded, irregular and degenerating forms (O) which go to make up all the parasites which are left prior to the total disappearance of the piroplasmosis.



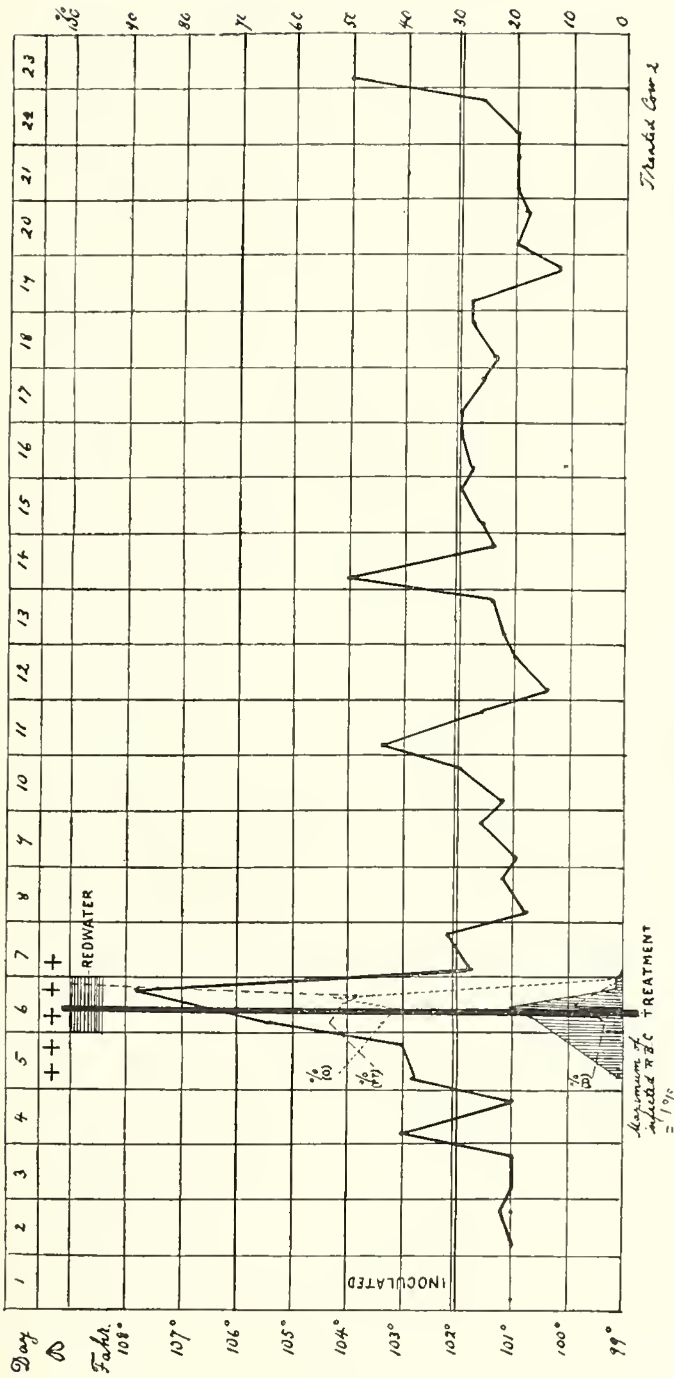
Control Cow I.



Control Cow II.



Treated Cow 1.



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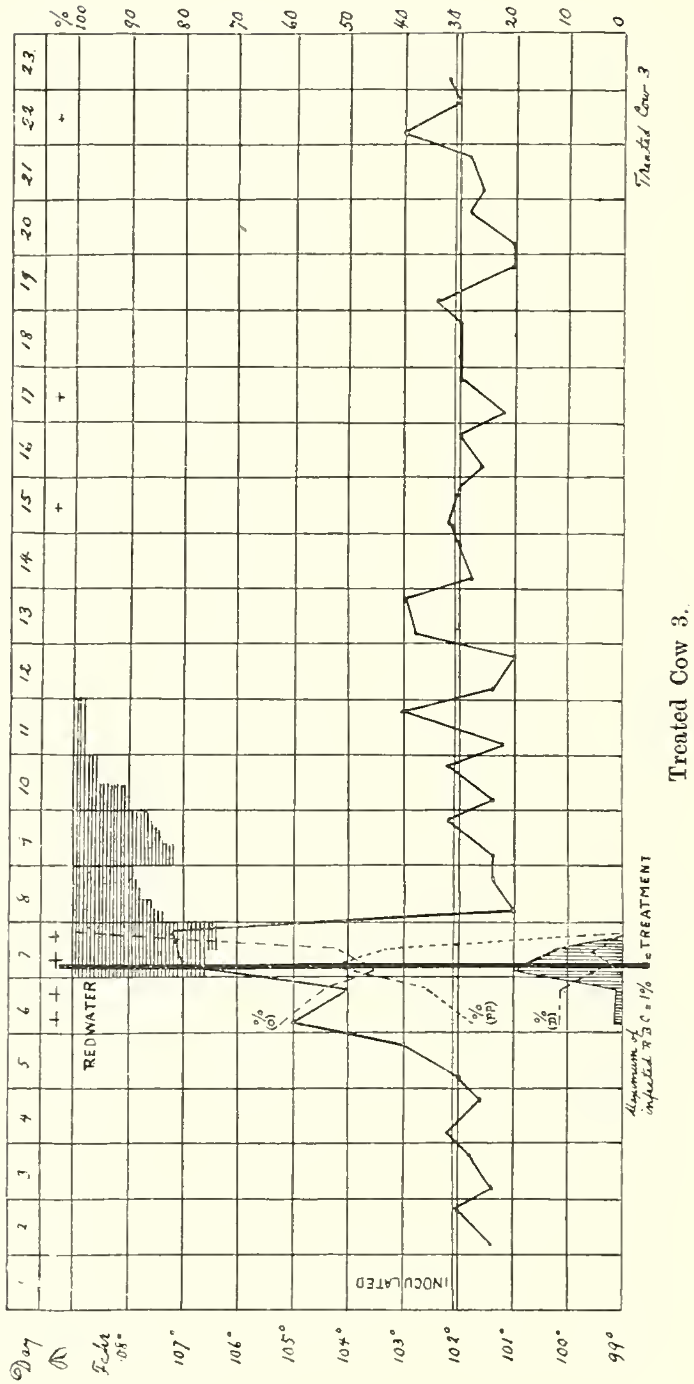

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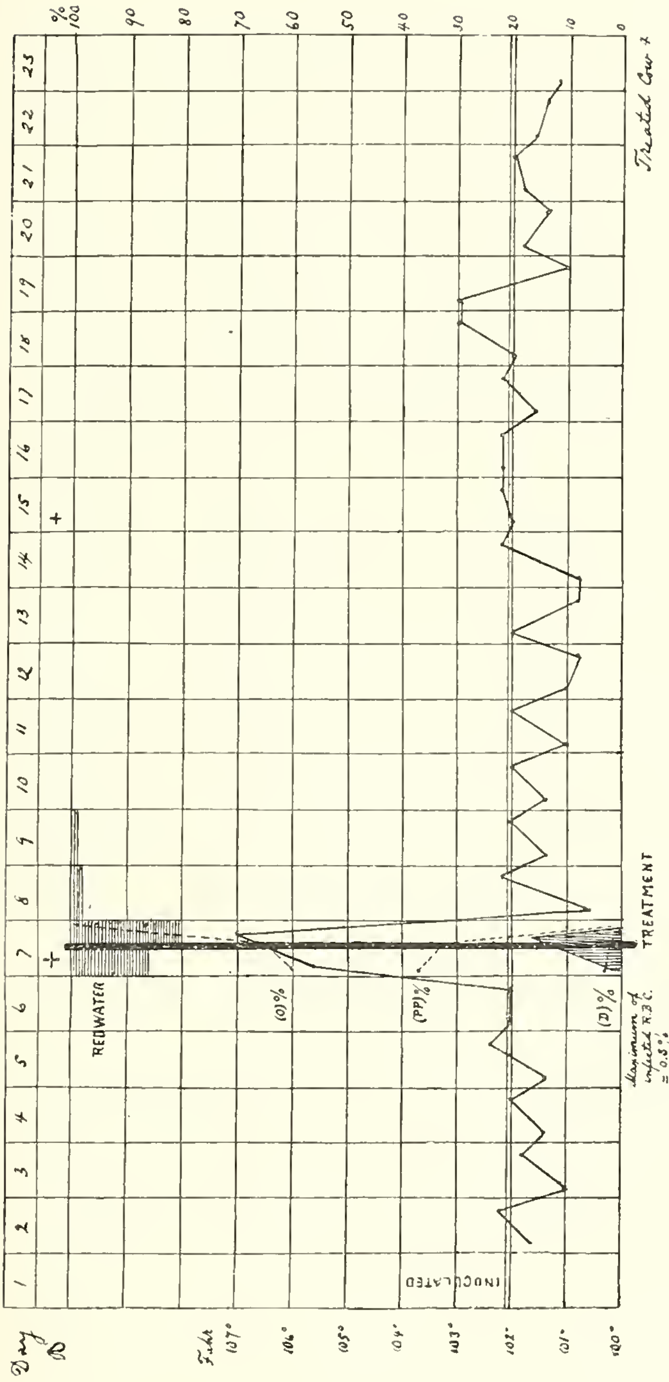

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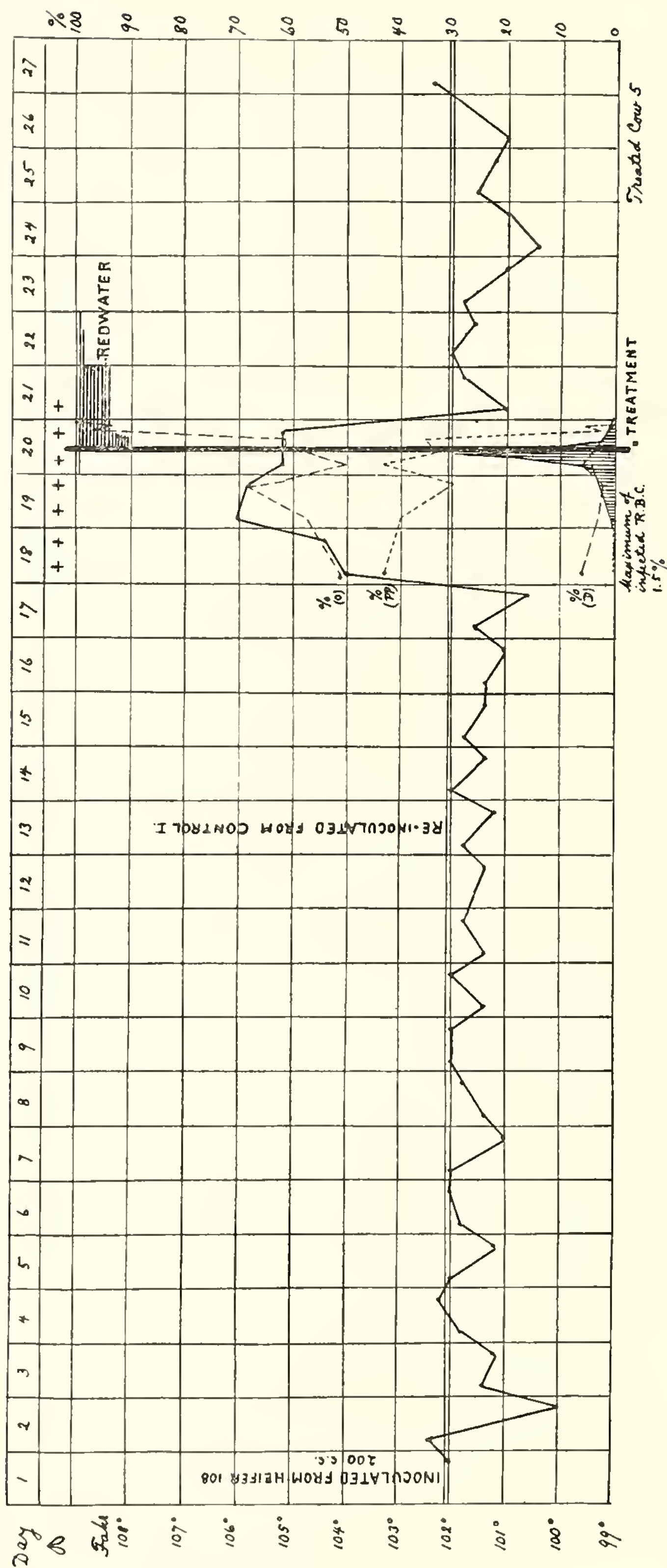

Treated Cow 5.

SUMMARY AND CONCLUSIONS.

1. Trypanblau promises to be an efficient remedy for bovine piroplasmosis, since it exerts a direct and obvious effect upon the parasites.

2. The effect of the drug upon *Piroplasma bovis* is similar to that which it produces upon the canine parasite. The dividing forms are the first to disappear, and after a few hours the pyriform parasites also disappear from the peripheral circulation; the parasites which are detected in the blood after a few hours appear degenerated and rounded or irregular; within nine to 45 hours or less all the parasites have disappeared from the blood.

3. As in canine piroplasmosis the disappearance of the parasites from the blood may be temporary. The parasites also disappear and reappear in small numbers (after two to 11 days) in animals undergoing natural recovery. In three treated animals the parasites reappeared in exceedingly small numbers after five to six days; in two they had not reappeared after 16 and 18 days respectively. The animals show no symptoms and progress towards recovery.

4. It remains to be determined (1) how long the blood of treated cows may contain parasites after the apparent recovery, (2) if the parasites in such recovered animals are altered in virulence, (3) if the parasites are capable of infecting ticks.

5. The experiments were conducted on nine cows, of which four served as controls and five were treated with trypanblau. Of the controls two suffered from haemoglobinuria, and one of these died of piroplasmosis; the two other controls had no haemoglobinuria and were very mild cases. All of the treated cows had haemoglobinuria and recovered. In four of the treated cows haemoglobinuria occurred before treatment began.

6. As might be expected, the drug exerts a more rapid effect when injected intravenously. The parasites disappear more slowly after subcutaneous injection of the drug. (Judging from our recent experiments on dogs, the giving of the drug *per os* promises to be without effect. See *This Journal*, p. 231.)

7. Although doses of 150—200 c.c. of a saturated watery solution of the dye were used, it is probable that smaller doses will prove efficient. The drug appears to produce no ill-effects upon cattle.

8. The drug, being a dye, has the disadvantage of colouring the tissues, more especially the subcutaneous connective tissues. How long the colouration persists remains to be determined. In any case this disadvantage can scarcely weigh in the balance as against saving the life of the animal, especially when used for breeding purposes.

9. We hope that experiments, which are about to be conducted in the field in Africa and elsewhere, will demonstrate the value of the remedy in practice.

10. Trypanblau and similar drugs should be given a trial in the treatment of Carçeag in sheep and Biliary Fever in horses.

Note regarding the solubility of Trypanblau.

In the course of our experiments we noted that crystals of trypanblau were frequently deposited after a short time even in 1 % solutions of the dye prepared with distilled water at room temperature. We therefore communicated with the manufacturers who had supplied us with the dye, Farbwerke vorm. Meister Lucius und Brüning, Hoechst a/M., desiring information regarding the solubility of trypanblau in distilled water. In a letter, dated 3 Aug. 1909, from the manufacturers, the latter state that they have made many experiments in this direction with the following results:

100 c.c. boiling water will dissolve 2 grammes of trypanblau and the solution can be easily filtered. On the other hand 5 grammes of the dye will not easily dissolve in 100 c.c. of boiling water and the solution filters badly, part of the dissolved dye separating in the filter; on cooling, the solution forms a gelatinous mass. The 2 % solution, after standing for 24 hours, has for the most part solidified into a gelatinous mass. In the case of 1 % solutions only a very small amount of the dye separates out after the lapse of 24 hours. A ½ % solution remains quite clear after standing for a long time. Owing to the difficulty of obtaining solutions of the dye and the tendency of the solutions to gelatinize on standing, it appears advisable to prepare the solutions immediately before use, in which case a 2 % solution at blood temperature could be used.

As stated we prepared our solutions in the cold, the dye being added in the proportion of 1 to 1·5 % to distilled water. Our solutions did not gelatinize, and there was always a certain amount of dye deposited. The deposit was fine and was mostly taken up in the syringe and injected with the solution. From a practical standpoint the 1 % solution appears to suffice and further experience may show that ½ % solutions are sufficiently effective. G. H. F. N.

OBSERVATIONS ON THE DIVISION OF SPIROCHAETES.

By DORIS L. MACKINNON, B.Sc.

(*From the Quick Laboratory, Cambridge.*)

2 Text-figures.

The morphology of the spirochaetes has proved in recent years a fruitful source of controversy. One has only to look through some of the vast literature on the subject to be convinced that the time has not yet come for drawing final conclusions, so varied and so contradictory are the observations by different well-accredited authors.

Concerning even such an elementary point as the mode of division, opinion is far from unanimous. In the well-worn dispute—are the spirochaetes protozoa or bacteria?—the supporters of the former view have almost all maintained that the division is longitudinal, while their opponents have as strongly insisted that it is transverse. One may be inclined to doubt whether the mere direction of division is really a point of very great value in such a discussion: as Schellack (1907) has recently pointed out, bacteria never divide by simple constriction as in all hitherto described cases of transverse division in spirochaetes[1]. Furthermore, it may be well to remember that transverse division is not the peculiar property of the bacteria but occurs among certain groups of protozoa also.

But apart altogether from systematic considerations, it is of interest to determine the mode of division in this group of organisms.

It was suggested to me that it would be useful to compile comparative summaries of the opinions of various observers on certain

[1] Swellengrebel (1907) described the formation of a sort of cell-wall between the dividing halves of *S. balbianii* just as in a bacterium—his observation has so far received no confirmation.

Comparative summary of evidence for longitudinal and transverse division in spirochaetes[1].

Spirochaeta	Observers of longitudinal division	Nature of observations	Observers of transverse division	Nature of observations
S. anodontae, Keysselitz ...	*Fantham ...	Longitudinal division seen *in vivo*.	...	...
	*Keysselitz ...	Observed *in vivo*. Longitudinal split divides organism into approximately equal halves, remaining at first attached throughout length by ectoplasm. Split proceeds from oue end to the other as the uniting ectoplasm disappears. Separating individuals move independently. Final act of separation takes some time.	Schellack ...	Transverse division described and figured from stained preparatious. Periplast becomes thinner at a middle point in the body—then disappears there, and spirochaete divides abruptly into two halves. There is no stage with loug drawn-out protoplasmic connection, and youug individuals after separation are correspondiugly blunt at one eud. In this respect division differs from that in blood spirochaetes[2].
S. balanitidis ...	*Hoffmann and Prowazek	Doubling of the "Periplastanhang," followed by a Y-stage, observed *in vivo*.	Schuberg ...	Records transverse division. Measurements of breadth are against possibility of longitudinal division.
S. balbianii, Certes ...	Certes ...	Normal method of division.	Borrel and Cernovodeanu	Transverse divisiou described.
			Certes ...	Transverse division also occurs.
	*Fantham ...	Usual method of multiplication. Splitting of basal granule, then of undulating membrane, finally of body. Usually the two halves separate by wriggling till 180° apart, but may remain at 30°–40° apart for a long time, executing very rapid movements. On two occasions forms watched *in vivo* for an hour: they died before division was complete.	Fantham ...	Trausverse division also occurs, but not so commonly as longitudinal. 1. Both long and short forms seen. 2. In stained preparations membrane occasionally discontinuous in centre. 3. Living specimens sometimes seen vibrating about a point possibly not central.
	Lustrac ...	Longitudinal division described and figured.	Lustrac ...	Transverse division suspected.

[1] Asterisks are placed before the names of such authors as state that they made observatious on the living organisms.

[2] Schellack (1909) also describes and figures similar transverse division in no less than eleven new species of spirochaetes from molluscs—*S. ostreae*, *S. chamae*, *S. spiculifera*, *S. modiolae*, *S. limae*, *S. cardii-papillosi*, *S. tapetos*, *S. acuminata*, *S. saxicavae*, *S. gastrochaenae*, *S. pusilla*. The conclusions were drawn from stained specimens.

S. balbianii, Certes (*continued*)	*Perrin ...	Normal method of multiplicatiou. Division of membrane; then longitudinal split resulting in compass-forms, making angle of 180°; final separation preceded by constriction first of periplast, then of contained plasma—separating individuals finally connected by strand of periplast only. Longitudiual splitting does not take long—this stage seldom seen: final separation takes longer—an individual at this stage watched for 40 mins., when it died.	Laveran and Mesnil	In stained preparations individuals showing great inequality in length—others in which the "gaîne" was discontinuous in the middle of body, others in which body was drawn out thin in this region, and (*in vivo*) some united two and two by a fine thread.
			Schellack ...	As in *S. anodontae* (*vide supra*). Final stages seen *in vivo*.
			Swelleugrebel...	Transverse division with a cell-wall as in *Spirillum gigantea*. Occasionally individuals seen constricted in the middle: author is doubtful whether these are dividing forms. Apparent longitudinal division simply parallel juxtaposition of two individuals: it may also be simulated by organisms bending on itself at a middle point.
S. buccalis, Cohu	...	...	Swellengrebel...	First sigu of divisiou is the formation in middle of cell of an abrupt curve, resembling a rupture. At this spot the cell begius to thin, and becomes paler: after formation of the two daughter-cells, the connecting filament is drawn out still fiuer, and fiually breaks.
S. culicis, Jaffé	Jaffé ...	In stained preparations one individual seen that might be interpreted as beginning of longitudinal divisiou.		...
S. dentium, Koch	Mühlens and Hartmann	Observed in stained specimens.	Mühlens and Hartmann.	Iu stained specimens, possibly transverse division.
			Zettnow	In stained preparations, iudividuals seen that might be interpreted as in process of transverse division.
S. duttoni, Breinl	Breinl ...	Occasionally met with, especially at stage of infection when parasites are disappearing from peripheral circulation. Scantiness of dividiug forms suggests that process of division is rapid.	Breinl ...	This the more usual mode of division. Parasites increase in length, become thinner in middle, and thinner part elongates till daughter halves separate.

Comparative summary of evidence for longitudinal and transverse division in spirochaetes (continued).

Spirochaeta	Observers of longitudinal division	Nature of observations	Observers of transverse division	Nature of observations
S. duttoni, Breinl (*continued*)	Carter ...	Y-forms seen in stained specimens: apparent equal division of chromatin granules.	Czaplewski ...	Records transverse division.
	Dutton & Todd	Stained specimens. Longitudinal division less frequent than transverse. Occurs generally towards end of attack, when parasites are disappearing from blood. Before division spirochaete increases in width, then a longitudinal split begins at one end, so that Y-forms are produced. Y-forms numerous in juice from splenic puncture at close of a fatal attack.	Dutton & Todd	Stained preparations. Perhaps most usual method of multiplication. Parasite increases in length, becomes thinner about middle, then is drawn out at this point till halves separate.
	Mayer ...	Apparent longitudinal division forms in stained preparations: especially abundant in smears from lungs.	Koch ...	Longitudinal division never seen. Where there is anything that can be interpreted as division, it is transverse.
			Levaditi ...	1. Spirochaetes frequently seen joined end to end in pairs by fine filament, which breaks at final separation. 2. Extraordinary inequality in length of individuals in same film.
			*Schellack ...	As in *S. recurrentis* (*vide infra*).
			*Zettnow ...	Studied both *in vivo* and stained. A long spirochaete divides in the middle—the two halves separating as the constriction thins out.
S. equi, Novy and Knapp	...	...	Martin ...	Some spirochaetes in stained preparations seen to have a paler and thinner portion in the middle.
S. hartmanni, Gonder	Gonder ...	Observed in stained specimens.	...	...

S. marchouxi, Nuttall (=*gallinarum*, Blanchard)	*Prowazek ...	1. Earliest stages difficult to make out. Dividing spirochaetes detected by their spasmodic movements. Before division the spirochaete increases in length and breadth. Division begins at one end; the split proceeds rather slowly through the length of the individual: the whole process once observed for a considerable time *in vivo*. 2. More common to see two spirochaetes still hanging together by a thin protoplasmic bridge—these are individuals in last stage of longitudinal division. 3. Presence of an undulating membrane and movements pointing to polarity in the cell, preclude possibility of simple transverse division.	Borrel ...	A constriction marks beginning of division: this is drawn out into a fine faintly-staining protoplasmic thread, uniting the two young individuals.
			Levaditi ...	A simple statement that division is transverse.
S. ovina, Blanchard	Martoglio and Carpano	A Y-form figured from a stained preparation.	Martoglio and Carpano	Observed in stained specimens.
S. pallida, Schaudinn	*Eitmer ...	Forms seen *in vivo* that might be interpreted as longitudinal division.	*Eitmer ...	Form seen *in vivo* that might be interpreted as transverse division.
	Gonder ...	Observed in stained specimens.		
	Krystallowicz and Siedlecki ·	Observed in stained specimens.		
			Prowazek ...	Dividing forms found in all preparations.
			*Schaudinn ...	Doubling of flagellum followed by rapid longitudinal splitting requiring only a few seconds. Process followed through *in vivo* in three cases. During division spirochaete becomes irregular in its curves—normal form recovered only when division is almost complete, and daughter spirochaetes are hanging together by their posterior ends. Dividing forms fairly numerous in stained preparations.

Comparative summary of evidence for longitudinal and transverse division in spirochaetes (continued).

Spirochaeta	Observers of longitudinal division	Nature of observations	Observers of transverse division	Nature of observations
S. pallida, Schaudinn (*continued*)	...	...	*Siebert ...	Dividing spirochaetes observed *in vivo* showed relatively rapid splitting to form Y-stage: then remained in this condition for some time. Marked tendency for rigid spiral form to be lost, curves becoming irregular till division complete, while wave-like movements over body are substituted for normal rotation round long axis.
S. pertenuis, Castellani	Berne ...	Described and figured.		
	Prowazek ...	Numerous very distinct longitudinal divisions seen.	...	...
S. pinnae, Gonder	Gonder ...	Longitudinal splitting very rapid. Begins with division of blepharoplast at anterior end. Equal division of chromatin to the two daughter cells.	Schellack ...	Transverse division as in *S. anodontae*—*vide supra*—studied on stained material.
S. plicatilis, Ehrenberg.	Schellack ...	Statement that the spirochaete divides longitudinally.	Zepf ...	Spirochaete breaks up into fragments.
			Laptschinsky...	Spirochaete fragments transversely: observed *in vivo*.
S. recurrentis, Lebert (=*obermeieri*, Cohn)	...	...	Anastasiades ...	Observed in stained preparations.
			Fränkel and Pfeiffer	Shown in figures of stained preparations.
			Norris, Pappenheimer, and Flournoy ...	Observations agree with those of Novy and Knapp.
			*Novy and Knapp	1. In stained preparations long spirochaetes frequently show pale transverse band in middle—suggestive of cell-wall. 2. Longitudinal division looked for daily both in living and in stained spirochaetes, but never seen. 3. *In vivo* long spirochaetes frequently seen to separate in two halves transversely. (This might be result of agglutination.) 4. In earliest stages of infection—before agglutination—long forms predominate. If division were longitudinal, short thick forms might be expected at this stage.

S. recurrentis, Lebert (=*obermeieri*, Cohn) (*continued*) ...	...	...	*Schellack ...	1. Extraordinary rarity of Y-forms, even in stained preparations, and the impossibility of distinguishing these from closely-apposed or agglutinated individuals. 2. Great constancy in thickness of individual spirochaetes. 3. Number of transverse divisions seen is quite in accordance with rapidity of multiplication. 4. Practically all spirochaetes of maximum length show a median constriction *in vivo*. 5. In final stage of division, the daughter spirochaetes, connected by a fine protoplasmic strand, are of a very constant length.
S. schaudinni, Prowazek (spirochaete of *Ulcus tropicum*)	*Prowazek ...	Observed repeatedly. Entire process of longitudinal splitting followed *in vivo*. During division, spirochaete becomes less active and moves spasmodically.		
S. vespertilionis, Novy and Knapp	*Gonder ...	Occurs both in blood of bat and in food-canal of tick. 1. Difficult to follow *in vivo*. A division already in Y-stage followed once for a short time. In stained preparations several division forms found. Longitudinal split begins at one end and spreads gradually to the other. 2. Not infrequently individuals seen hanging together by their ends—probably final stage of longitudinal division, but interpreted as transverse division by Nicolle and Comte. 3. Agglomeration never seen, and very rare to see spirochaetes twisted together.	Nicolle & Comte	The daughter-cells remain for some time connected by their ends.
S. vincenti ...	...	..	Mühlens ...	"Apparently transverse."

questions connected with the morphology of spirochaetes. The literature is so extensive, and the observations so scattered, that a résumé of this sort may make it easier to weigh the pros and cons. The preceding table deals thus with the evidence for longitudinal and transverse division in spirochaetes. It does not pretend to be exhaustive, but it includes the chief statements of the best-known authorities.

In comparing the results given above, I was struck by the fact that the conclusions were so seldom based on observations of the living organism. It is not always clear from authors' statements whether they confined their study to stained preparations, but, so far as I can gather, the *entire* process of longitudinal division has been observed *in vivo* only by Prowazek (*S. marchouxi*, spirochaete of *Ulcus tropicum*, *S. balanitidis*)[1], Schaudinn (*S. pallida*), Siebert (*S. pallida*), and Keysselitz and Fantham (*S. anodontae*)[2], while Schellack (*S. recurrentis, S. duttoni*), Novy and Knapp (*S. recurrentis*) and Zettnow (*S. duttoni*) are the only observers of transverse division from its first indication till the separation of the daughter parasites. This seems strange, since stained preparations are, in a case like this, notoriously unreliable: and so long as these are used as sole evidence, just so long will the supporters of the view that spirochaetes are bacteria insist on regarding the Y-forms as agglutinations, and the supporters of the view that spirochaetes are protozoa will see in the so-called transverse divisions simply the final stage of longitudinal division.

Personal observations on the division of *Spirochaeta recurrentis*.

Professor Nuttall kindly suggested that I should try to make some observations on the division of *S. recurrentis* (Russian strain) *in vivo* from the blood of infected mice. At first I examined, at room temperature, very thin films of fresh infected blood, sealed with vaseline. Later on I employed a warm stage (Nuttall thermostat), with a

[1] Prowazek (1908), further states, but without particulars, that he has observed longitudinal division in all the smaller spirochaetes, with the exception of *S. dentium*, *S. vincenti*, *S. recurrentis* (European variety), spirochaetes from the intestines of the dog and the cat, spirochaetes from an abscess on the lower jaw of a chimpanzee, and *S. plicatilis*. Keysselitz and Blanchard also express their opinion that the spirochaetes as a group are characterized by longitudinal division.

[2] Perrin and Fantham both observed longitudinal division in *S. balbianii* up to the final stage when, however, the individuals died before having separated.

temperature of 37° C., and I found it more convenient to use the serum only of citrated blood[1].

For the first half hour or so after being put into the warm stage, the spirochaetes were so active that it was impossible to follow any one individual. Gradually they slowed down somewhat, and it was at this stage that I made my observations. Later on, when they became still more sluggish, agglutination produced appearances often deceptively like divisions, which had to be discounted. Even when it was not a question of actual agglutination, the tendency of the spirochaetes to twist together for a short time in groups of two or three, made it very difficult to keep any one separately in view. At first also I lost many among blood-corpuscles.

I. *Longitudinal Division.* On two occasions only did I see what I felt convinced were spirochaetes in process of longitudinal division.

(1) The first was an individual that caught my attention by the character of its movement, which was slower and more spasmodic than that of the other spirochaetes in the same field. I then saw that it was apparently splitting longitudinally, and had already reached the Y-stage. The splitting portions were connected through the first two curves of their length, and then diverged at an acute angle: one individual had about seven curves free, the other was rather shorter. A certain amount of independent movement was shown in the two halves, one moving more rapidly or more slowly than the other; frequently they lay parallel, and twisted round one another, their curves coinciding or opposite. During the first fifteen minutes the spirochaete showed scarcely any appreciable forward movement, then it moved across the field, unsplit end first. Shortly before it was finally lost to sight—after having been watched for nearly 40 minutes—the split appeared to have extended over another curve. (Fig. 1 *a*, *b*, *c*, *d*.)

(2) An individual, apparently in an advanced Y-stage of longitudinal division, was kept in sight for seven minutes. The two halves

[1] A simple method of obtaining small quantities of serum full of spirochaetes but almost free of blood corpuscles, is the following:—Draw up into a fine pipette, *with an opening narrower than the general lumen of the tube*, a few drops of citrated blood. Lay the pipette horizontal for a short time; the blood corpuscles will settle in a layer in the under half of the lumen of the tube below the level of the opening, while numerous spirochaetes remain in the clear serum above. Then, still keeping the pipette nearly horizontal, blow out a minute drop of the serum on to a slide. This method has the advantage of avoiding possible injury to the organisms through centrifugalizing, and makes it easy to employ very small quantities of blood at a time.

were in this case approximately equal, and the movement, though rather spasmodic, was more rapid than in (1).

II. *Transverse Division.* I was struck by the fact that in most spirochaetes that had attained considerable length—9 to 14 curves—there appeared a weak point, about half-way along the organism, at which bending occurred, as the arm bends at the elbow-joint. In some cases there seemed to be a distinct constriction at this point.

On eleven occasions I saw what I should interpret as transverse division.

1. An individual with a thin drawn-out portion in the middle was seen to separate into two at that point.

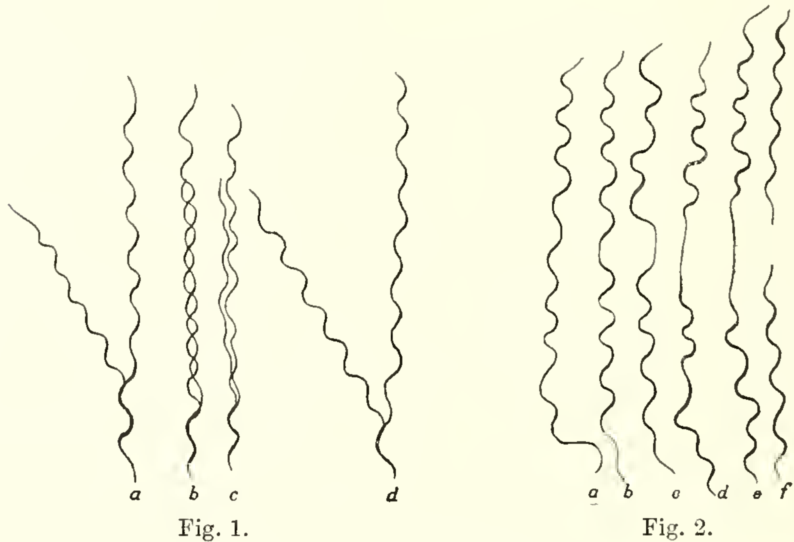

Fig. 1. Fig. 2.

2. Two short spirochaetes joined only by a fine protoplasmic bridge were watched for some time, during which the connection thinned out till they separated. This appearance has been aptly likened by Schilling to the drawing out of a glass tube in a flame.

3. A long individual, with approximately 14 curves, showed a thin zone half-way along its length. It was watched for 20 minutes. During this time the thin portion became more and more drawn out and the two halves separated, after spasmodic and "purposeful" wrenching movements.

4. Two short individuals—one rather longer than the other—connected by a fine strand, separated after having been watched for several minutes.

5. A long individual (about 12—14 curves), with a thinner median portion was watched for 20 minutes. Its movements were agitated and

jerky, but it did not change its position much. The thin portion became finer and finer, till its presence could scarcely be suspected, but for the simultaneous tugging movements of the separating halves. Finally the connecting thread broke, and the daughter-spirochaetes departed in different directions.

6. A long spirochaete was seen, in which the two halves were separated by a finely drawn out middle portion.

7. A spirochaete with a thinner median portion was observed for 20 minutes. During that time the middle portion became longer and finer till scarcely visible. Death occurred before complete separation took place.

8. A long spirochaete was noticed that showed very marked bending at a slightly narrower portion half-way along its body. This portion was watched thinning out till the connection between the daughter halves became scarcely perceptible: they died after 20 minutes, and before they had completely separated.

9. A long spirochaete with a weak central point was followed for about 10 minutes, during which time the median portion had thinned out considerably.

10. A long spirochaete—about 14 curves—was followed for 30 minutes. During that time the rather narrower median portion became still thinner, till the two halves were widely separated, though still attached by a fine protoplasmic bridge. At this point the spirochaete became gradually immobile and died.

11. A long individual was kept in sight for about 25 minutes. Shortly after the beginning of the observation, a median thin portion became visible: division at this point went forward rather rapidly and the two halves separated. (Fig. 2 *a*, *b*, *c*, *d*, *e*, *f*.)

Conclusions.

In two cases apparent longitudinal division was seen in *Spirochaeta recurrentis*: in eleven cases the division was apparently transverse. It is not possible to say with absolute certainty, except in one instance, that these transverse divisions were not the final act in a longitudinal division. I am nevertheless inclined to think *that both forms of division may take place, transverse being the more common.* Breinl, and Dutton and Todd have expressed the same opinion with regard to *S. duttoni*, and Fantham also states that in *S. balbianii* both modes of division occur.

I should like to express my thanks to Professor Nuttall for his kind suggestions and help, and to the Carnegie Trust for the grant which has made my work possible.

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OBSERVATIONS ON THE EFFECT OF VARIOUS CHEMICAL REAGENTS ON THE MORPHOLOGY OF SPIROCHAETES.

By DORIS L. MACKINNON, B.Sc.

(*From the Quick Laboratory, Cambridge.*)

4 Figures.

It has been stated[1] that in the stomach of the bed-bug the spirochaetes of relapsing-fever may frequently be observed to lose their normal regular curves and become more or less "worm-like." This is of interest to those who oppose the view that spirochaetes are rigid spirals, a view which, after gradual abandonment by most observers, has lately been again advanced by Novy and Knapp in their study on *S. recurrentis.*

It seems probable that the alteration in form may be due to the action of some constituent of the digestive juices of the bug. Professor Nuttall suggested to me that it might be possible to produce the same effect artificially by treating the living organism with various chemical reagents. In this attempt I was not altogether successful. Certain acids (notably formic acid) and quinine, and, in a lesser degree, glycerine, did cause a straightening out of the body and a corresponding flattening of its curves; but the appearances could scarcely be described as "worm-like." They help, however, to combat the notion that the curves are rigid.

In addition to the liquids just mentioned, and certain other drugs and acids, I subjected *S. recurrentis* to some of the reagents used by previous observers on various spirochaetes in their attempts to decide the systematic position of these organisms.

I employed *S. recurrentis* (Russian strain) in thin films of infected mouse-blood, mixed with equal parts of citrated salt solution. As a rule,

[1] Löwenthal (1905, Die Spirochaeten, *Biophysik. Centralbl.* I.) quotes Schaudinn's observations on this point. Klodnitsky (1907, *Centralbl. f. Bakt.* XLV. pp. 126–8) claims to have seen the same phenomenon. Nuttall has repeatedly observed it himself, but has expressed the opinion (1908, *Parasitology*, I. p. 144) that Klodnitsky's photographs show that that author was dealing with the spermatozoa of the bug, and not with altered spirochaetes.

the reagent was diluted (with citrated salt solution where possible) in about six graduated strengths, and these were allowed to diffuse in minute quantities below the cover-glass, the effect of each dilution being carefully watched on the living organism. Care had to be taken to prevent the diffusion currents from being too violent, as these were apt to distort the spirochaetes mechanically, and so vitiate the results.

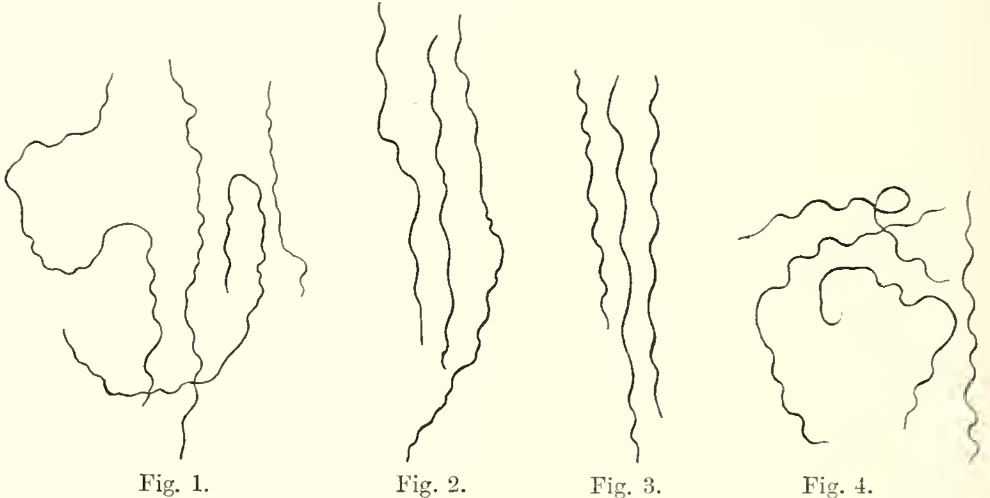

Fig. 1. Fig. 2. Fig. 3. Fig. 4.

The following table gives the results of my observations:

Reagent employed	Effect on *Spirochaeta recurrentis*
1. Distilled water ...	Spirochaetes gradually immobilized—by the end of a few minutes all at rest. There was a strong tendency to collect in tangled skeins. Occasionally an individual showed flattening of its curves. There was marked blurring of outlines. Distilled water after 25 % salt solution produced no effect.
2. Sodium chloride (1 % to 40 %)	All solutions immobilized (in the stronger solutions, instantly), but produced no other effect beyond giving the spirochaetes a more refractive appearance. Unchanged after 16 hours.
3. Caustic potash ... (5 % to 25 %)	Majority of spirochaetes dissolved. Some remained as pale shadows.
4. Hydrochloric acid (·01 % to 10 %)	All dilutions produced instant immobilization. The spirochaetes had their curves much flattened out, or even reduced to minute irregular "crimping": there was also a tendency to coil at the ends, and they were often twisted together in parallel bunches. They became paler, but were not dissolved after 16 hours. (Fig. 1.)
5. Nitric acid ... (2 %)	Produced much the same effect as hydrochloric acid. On the whole, the spirochaetes seemed less inclined to curl and twist up.
6. Citric acid ... (·01 % to 10 %)	Spirochaetes at once immobilized: there was a tendency to run together in bunches: they also became paler and showed occasional flattening of curves. Undissolved after 6 hours.
7. Formic acid ... (·02 % to 40 %)	Instant immobilization: curves frequently much flattened out: occasional tendency for curling over at the ends: many become

Reagent employed	Effect on *Spirochaeta recurrentis*
	much paler. After 18 hours no further change, except that in the stronger solutions the spirochaetes often appeared very pale and shadow-like.
8. Alcohol ... (5 % to 100 %)	Immobilization instant in the stronger solutions—in solutions below 30 % this was more gradual. No appreciable change in form, beyond occasional slight flattening of curves.
9. Glycerine ... (5 % to 85 %)	Immobilization complete in about a minute or two in the weaker solutions: above 25 % it was almost instantaneous. The form was very little altered, except for occasional marked flattening of the curves. No further change after 23 hours. (Fig. 2.)
10. Quinine (sulphate) (·01 % to 10 %)	Instant immobilization. The curves became much flattened out: frequently the spirochaetes were almost straight, or minutely "crinkled." This was particularly marked in the stronger solutions, but was noticeable in all. (Fig. 3.)
11. Soamin (1 %) ...	Much the same effect as in distilled water. A few spirochaetes still moving after 10 minutes, but only very sluggishly. No alteration after 16 hours.
12. Arsacetin (5 %)...	Some immobilized at once: the majority continue to move sluggishly for a few minutes. The general appearance was much as in distilled water. No alteration after 16 hours.
13. Trypanblau (1 %)	Much the same effect as in distilled water. A stronger tendency for the spirochaetes to form intertwining bundles: frequently the individuals forming the groups were much twisted upon themselves. 20 hours later, no alteration. (Fig. 4.)
14. Trypanrot (2 %)	Same as in (13).

CONCLUSIONS.

The form of *Spirochaeta recurrentis* is not appreciably altered by immersing it in distilled water, NaCl solutions, alcohol, soamin, arsacetin, trypanblau, trypanrot. Flattening of the curves of the spiral produced by glycerine, citric acid, formic acid, nitric acid, quinine and hydrochloric acid —this effect is most marked in quinine and hydrochloric acid. In several of the reagents employed—notably quinine, HCl, trypanrot and trypanblau and in distilled water—there is a distinct tendency for the spirochaetes to curl up at their extremities, and to become intertwined in bundles. There is no evidence of plasmolysis. The spirochaetes dissolve readily in caustic potash: the acids employed tend to make them become paler. All the reagents produce rapid immobilization.

For the sake of comparison with the above, and in order to bring together into a convenient form observations scattered throughout the literature, I have drawn up a tabular summary of the results of previous experiments similar to mine[1].

[1] The list contains all the chief references, but cannot pretend to be exhaustive.

Comparative summary of observations on the effect of various chemical reagents on the morphology of Spirochaetes.

Reagent	Spirochaeta	Effect on Spirochaete	Observer
1. Distilled water	*S. balbianii*, Certes ...	Death occurs: plasma swells and bursts periplast	Perrin.
		Die quickly	Fantham.
	S. culicis, Jaffé ...	Die quickly	Jaffé.
	S. marchouxi, Nuttall (= *gallinarum*, Blanchard)	(After 10 $^0/_0$ NaCl solution.) No bursting out of plasma through periplast	Prowazek.
	S. pallida, Schaudinn	Die quickly	Eitmer.
	S. recurrentis, Lebert	Increased activity for 6–8 hrs.: then become sluggish: after coming to rest, their form is perfectly retained: revived by 5 $^0/_0$ NaCl solution[1]	Novy and Knapp.
2. Tap-water ...	*S. balbianii* ...	Live slightly longer than in distilled water	Fantham.
3. Sea-water ...	*S. anodontae*, Keysselitz	Lived one hour	Fantham.
4. Sodium chloride	*S. balanitidis* ..	10 $^0/_0$ solution causes spirochaetes to appear narrower and more refractive: no plasmolysis	Hoffmann and Prowazek.
	S. marchouxi ...	5–10 $^0/_0$ solutions had no effect beyond occasional production of a protoplasmic "bead"	Prowazek.
	S. pallida ...	Swell: fraying repeatedly seen, also knot-like thickenings	Siebert.
	S. vespertilionis, Novy and Knapp	2–5 $^0/_0$ solutions produce no plasmolysis	Gonder.
	Spirochaetes of the mouth	10–12 $^0/_0$ solutions produce shrinkage here and there, through extraction of water: tendency to form tangled skeins	Siebert.
5. Sodium carbonate	*S. buccalis* ...	In saturated solution are unaltered ...	Swellengrebel.
	Spirochaetes of the mouth (*S. buccalis*, etc.)	Become paler	Hoffmann and Prowazek.
6. Caustic potash[2]	*S. balanitidis* ...	Become paler and fewer: after 2 hours all disappeared	Hoffmann and Prowazek.
	S. balbianii ...	In 10 $^0/_0$ solution some retained shape for a long time and were not dissolved. (Did not appear blue in iodine and sulphuric acid after caustic potash)	Fantham.
	S. buccalis ...	Dissolve	Hoffmann and Prowazek.
		In 10–20 $^0/_0$ solutions become paler ...	Swellengrebel.
	S. culicis	Dissolve in 1 $^0/_0$ solution	Jaffé.
	S. marchouxi ...	Die; some dissolve, others remain as pale "shadows"	Prowazek.
	S. pallida ...	Dissolve	Eitmer.
	S. vespertilionis ...	Die and are dissolved	Gonder.

[1] I fail to understand how Novy and Knapp arrived at this result. I repeatedly tried the effect of distilled water on the spirochaetes, and always found that they became immobile in a very few minutes.

[2] Thesing tried the effect of caustic potash on "various spirochaetes," and found that they remained unaltered.

Reagent	Spirochaeta	Effect on Spirochaete	Observer
7. Eau de Javelle	*S. buccalis*, etc. ...	No effect	Hoffmann and Prowazek.
	S. marchouxi ...	No effect at first, but after a time become knotted and twisted up	Prowazek.
8. Hydrochloric acid (dilute)	*S. buccalis*, etc.	Swell somewhat	Hoffmann and Prowazek.
		Become paler	Swellengrebel.
9. Nitric acid (dilute)	*S. buccalis*, etc. ...	Dissolve	Hoffmann and Prowazek.
		In 1 : 5 solution become paler ...	Swellengrebel.
10. Sulphuric acid (concentrated)	*S. balbianii* ...	Periplast dissolved after 3 hours: undulating membrane not especially soon dissolved: nuclear core remained	Fantham.
11. Acetic acid (strong)	*S. balbianii* ...	Not dissolved: retain shape for long time: a certain amount of fraying	Fantham.
12. Acid carb. liquefact	*S. marchouxi* ...	No effect. (Other acids produce no effect either)	Prowazek.
13. Iodine ...	*S. balbianii* ...	Stain brown: in iodine + concentrated sulphuric acid stain light yellow, and retain shape for long time	Fantham.
	S. marchouxi ...	Stain yellow like bacteria	Prowazek.
14. Sublimate[1], Lysol, and other antiseptics	*S. buccalis*, etc. ...	In antiseptics break up into numerous short individuals	Wechselmann and Löwenthal.
	S. pallida ...	Become immobile	Eitmer.
		In sublimate (1:1000) are killed, but not dissolved	Prowazek.
		In sublimate (1:1000) all spirochaetes in tissues are not in 10 % solution, no swelling or maceration, but become thin and strikingly paler	Siebert.
15. Alcohol ...	*S. pallida* ...	In 30 % die quickly	Eitmer.
16. Glycerine ...	*S. balanitidis* ...	Die quickly without losing curves, though these become somewhat flattened	Hoffmann and Prowazek.
		Become immobile	Müller and Scherber.
	S. marchouxi ...	In 40 % solution majority contract, some die, while others take on peculiar tangled form, but continue mobile. (Still infective after 12 hours)	Prowazek.
	S. pallida ...	Some not killed, but altered to spindle-shaped forms	Schaudinn.
		Even in 10 % solution spirochaetes stretch out quickly, lose their mobility and remain in this condition; NaCl solution fails to revive them, and they are uninfective	Eitmer.
	S. vespertilionis ...	Do not live long: stretch out, or first stretch and then contract together	Gonder.

[1] Wechselmann and Löwenthal also state that the mercury treatment causes *S. pallida* to break up into fragments.

Comparative summary, etc. (continued).

Reagent	Spirochaeta	Effect on Spirochaete	Observer
17. White of egg	*S. balbianii* ...	Die very quickly	Perrin.
		Die very quickly	Fantham.
18. Pepsin + HCl	*S. buccalis*, etc. ...	Some swell up, some are practically dissolved, others remain unaltered	Siebert.
	S. pallida ...	Contents of periplast apparently dissolved	Prowazek.
		Swollen appearance and partial paling	Siebert.
19. Sodium taurocholate	*S. buccalis*, etc. ...	Dissolve instantly	Neufeld and Prowazek.
	S. marchouxi ...	Dissolve instantly	Neufeld and Prowazek.
	S. pallida ...	Dissolve in 10 % solution	Prowazek.
		Dissolve	Neufeld and Prowazek.
		In 10 % solution first swell, then gradually dissolve	Siebert.
	S. recurrentis ...	Dissolve	Neufeld and Prowazek.
	S. schaudinni (Spirochaete of *Ulcus tropicum*)	Dissolve	Prowazek.
20. Sapotoxin ...	*S. buccalis*, etc. ...	Become immobile and die	Neufeld and Prowazek.
	S. marchouxi ...	Become immobile and die	Neufeld and Prowazek.
21 Saponin ...	*S. pallida* ...	In 10 % solution become swollen and show partial paling	Siebert.

Conclusions.

The general consensus of opinion goes to show (1) that spirochaetes are dissolved in solutions of caustic potash (5 to 25 %) and of sodium taurocholate (10 %), and possibly to a slight extent in acids; (2) that they are not plasmolyzable in the strict sense, and that plasmotypsis is rare; and (3) that certain reagents, such as glycerine, may alter the form, by flattening the curves, or by causing contraction.

I am greatly obliged to Professor Nuttall for his suggestions in connection with these observations. The work was done during my tenure of a research grant from the Carnegie Trust for the Scottish Universities.

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NOTE ON TWO NEW FLAGELLATE PARASITES IN FLEAS—*HERPETOMONAS CTENOPHTHALMI*, N. SP., AND *CRITHIDIA HYSTRICHOPSYLLAE*, N. SP.

By DORIS L. MACKINNON, B.Sc.

Plate III.

(*From the Quick Laboratory, Cambridge.*)

There seems little reason to doubt that most blood-sucking invertebrates harbour in their digestive tract natural flagellate parasites, which have erroneously been described by various authors as part of the life-cycles of blood-parasites from vertebrates. Among arthropods such natural flagellates have now been observed in ticks, lice, bugs, mosquitoes, biting and non-biting flies, and fleas; and the list of such hosts steadily grows.

The following have been recorded from fleas:

(1) *Herpetomonas*, sp., Balfour (1906) (= *Crithidia pulicis*, Wenyon (1908)), from *Loemopsylla cleopatrae* "and possibly other species."

(2) *Herpetomonas* (?), Swingle (1907), from rat-fleas.

(3) *Crithidia*, sp., Patton (1908), from the larvae of *Ctenocephalus felis*.

(4) *Crithidia ctenophthalmi*, Patton and Strickland (1908), from *Ctenophthalmus agyrtes*.

I propose to describe here two new intestinal flagellates from two species of fleas, *Ctenophthalmus agyrtes* and *Hystrichopsylla talpae* (or possibly *C. bisoctodentatus*)[1]. In naming them *Herpetomonas ctenophthalmi* and *Crithidia hystrichopsyllae* respectively, I have been guided by the definition of these genera in a recent paper by Patton and Strickland in this *Journal*. My thanks are due to Professor Nuttall

[1] The Hon. N.C. Rothschild has kindly identified these fleas.

for his kindness in putting the material at my disposal, and to the Carnegie Trust, from which I received a research grant during my work in Cambridge.

The material for dissection consisted of 29 adult fleas taken from three moles' nests supplied from Tring Park, through the courtesy of the Hon. N. C. Rothschild. The nests were sent to the laboratory on the 9th of February. I did not begin dissection until the 23rd of February, and the last infected flea was found on the 20th of March: I record this fact, because it shows that the fleas had been long without food, and that therefore the possibility of the parasites' having come from infected mole-blood was small.

Of the twenty-nine fleas examined, four were found infected with flagellates.

The method of observation of the parasites was very simple. The digestive tract of each flea was dissected out in a drop of normal salt-solution, and examined under a $\frac{1}{6}''$ objective. If there were any parasites present, the flagellates could always be made out inside the gut, moving in a peculiar jerky manner, aptly described by Balfour as resembling that of the agitated needle of a compass. Parasites were never seen in the mid-gut. They were chiefly congregated in the expanded upper portion of the hind-gut into which the malpighian tubes open. They could also be seen in little groups lower down; frequently the gut was slightly dilated at these points. The cysts, though usually present in the rectum, could not be seen in the living state, owing to the opacity of the large rectal glands. I much regret that scantiness of material prevented my making a proper study of the living parasites.

When the flagellate had been located, the gut was divided into three portions, and smeared on a slide. In three instances the smears were then dried, and fixed in absolute alcohol. I obtained a better result with my last specimen, which I fixed in the manner recommended by Swingle, *i.e.* fix with vapour of 40 % formalin for 10 minutes, and then wash off any superfluous salt with warm water: after that, I fixed further in alcohol for 10 minutes. All the preparations were stained with Giemsa's stain.

I. *Herpetomonas ctenophthalmi*, n. sp.

Three fleas were infected. One belonged undoubtedly to the species *C. agyrtes*; the other two were either *C. agyrtes* or *C. bisoctodentatus*.

The first contained relatively few parasites, and these mostly encysting or encysted; the other two showed a much richer infection, with numerous flagellate forms in the upper reaches of the hind-gut. Patton has divided the life-cycle of such organisms into three stages, pre-flagellate, flagellate, and post-flagellate. I propose to follow the same order in my description.

(1) *Pre-flagellates.* I found no such stage, all the parasites in the upper part of the hind-gut being already adult flagellates or forms beginning to encyst. This points to the possibility that the early stages previous to flagellation may be gone through in the larva, and until the larvae and pupae are examined, the account of the life-cycle must remain incomplete.

(2) *Flagellates.* By far the greater proportion of the parasites were in this stage. There were a few free forms with intact flagellum (Plate III, Fig. 1), but the majority were grouped in radiating masses, attached to one another in the centre of the rosette by the reduced flagella (Plate III, Fig. 3). The form of the adult flagellate is extremely long and attenuated ($21\mu \times 2\mu$, without reckoning the flagellum), tapering posteriorly to a mere filament. The *nucleus* is usually in the anterior half of the body: it is oval to round in form (about 1μ in diameter) and stains purplish-red throughout, with a few minute darker granules. The *blepharoplast* lies anterior to the nucleus, usually at about a distance of 1 to 2μ, but may be closely apposed to its anterior border. The form is a blunt rod, but may be dumb-bell-like. The *flagellum* is complete only in a few instances; in favourable specimens it can be traced back almost to the blepharoplast as a faintly reddish streak: in one instance it could be seen to originate in a minute, red-staining granule, slightly in front of the blepharoplast. In most cases the parasite forms one of a group, or has attached itself to the gut-wall preparatory to encystment, and the flagellum has become very short, or is reduced to a pink-staining mass of apparently viscous matter (Plate III, Fig. 2 *a*, *b*, and *c*). The *cytoplasm* stains delicate lavender to purplish. The portion behind the nucleus is usually highly vacuolated, and sometimes contains a few small chromatin-like granules. Anterior to the nucleus it stains more deeply, taking on a pink colour in the neighbourhood of the flagellum. Between the nucleus and blepharoplast there is sometimes a vacuole.

The great variability in size of the flagellates is very striking. Alongside the larger forms occur small forms, measuring only about $7\mu \times 1\mu$, or even less. This may possibly be accounted for by the fact that

unequal division is common. Such a case is that of Plate III, Fig. 4 *b*, where a thin cell, containing two minute dark-staining granules and an anterior vacuole, is being split off from the side of an encysting individual.

In one flea the flagellates presented a somewhat different appearance (Plate III, Fig. 4 *a* and *b*). They were shorter and broader ($15\mu \times 3\mu$, on an average) and less attenuated posteriorly; but the main difference consisted in the distribution of the chromatin, which crowded the cytoplasm in irregular strings and granules. These stained deep red to purplish, and it was often impossible to distinguish the blepharoplast. The nucleus, on the other hand, stained with great difficulty, and generally appeared as a pale area surrounded by "chromidia" (?).

(3) *Post-flagellates.* These were found mainly in the rectum and occur singly or in groups (Plate III, Fig. 8). The parasites shorten and broaden, becoming pear-shaped and finally round (Plate III, Figs. 5 to 11). They also increase considerably in volume ($5\mu \times 4\mu$ is an average measurement). The *nucleus* undergoes interesting changes, the meaning of which is not at all clear. In the earlier stages of encystment it swells out (2μ), and no longer stains uniformly: the general mass is pale pinkish-red, with deeply-staining chromatin collected round the borders, and connected by irregular granules with a central mass (karyosome?) (Plate III, Figs. 7 and 8). In some instances where the nucleus appears to be undergoing division, the chromatin is collected into a uniformly-staining mass, which becomes dumb-bell-like and divides by simple constriction (Plate III, Fig. 14 *a*). Disintegration of the nucleus may occur. Fig. 12 shows a cyst in which the nucleus has taken on a blurred and fragmentary appearance. In Fig. 13 the whole nuclear content is being scattered into the cytoplasm as chromidia. Finally, there are a few individuals which have apparently lost all but a trace of the nucleus—such forms are shown in Fig. 14 *b* and Fig. 15. The cysts that have completely rounded up generally show an evenly-staining nucleus of smaller size than in the initial stages of encystment, and with the chromatin peripherally disposed (Fig. 11). The *blepharoplast* becomes broader and stains more lightly—sometimes the nucleus and the blepharoplast stain the same shade. Its position in the cell is variable, but in the pear-shaped forms it usually lies against the nucleus, either anterior or to one side. In the final stage of rounding-up it may be widely separated from the nucleus (Fig. 11). Occasionally cysts are seen in which the blepharoplast is undergoing repeated division (Fig. 12). This may reach a condition in which the blepharoplast is

apparently broken up into a number of small granules scattered through the cell. The *flagellum* is never seen "cast off." The distal portion which attaches the parasite to the wall of the gut becomes reduced to a little circular mass of pink-staining material, persisting through the early stages of encystment: later on this is completely absorbed. The portion of the flagellum within the cell sometimes shows as a faintly reddish streak running down towards the blepharoplast, but more often is simply indicated by a pink-staining area. All trace of this disappears in the later stages. The *cytoplasm* shows great variability in its staining properties. Early in the encystment it stains a pale, clear blue and is markedly free from granules: in the neighbourhood of the flagellum-root it has a pinkish tint. Other cysts stain a deep purplish-pink, and contain minute darker-staining granules. Generally there are one or two large, indefinitely-outlined vacuoles, but the later stages often show numerous, small, round vacuoles all through the cell (Fig. 11). In no instance was anything seen resembling a well-developed cyst-wall, such as Prowazek and other authors have described in the "Dauercysten" of *Herpetomonas*.

II. *Crithidia hystrichopsyllae*, n. sp.

Only one flea was infected with this parasite. The hind-gut contained numerous encysting stages, and, judging from these alone, it would be impossible to say that one was not dealing with a *Herpetomonas*. But though free adult flagellates were few, they were unmistakeably crithidian in character.

(1) *Pre-flagellates.* I saw no stage that could be so interpreted.

(2) *Flagellates.* The adult flagellates vary extraordinarily in size, and in general appearance. The following are the chief types: (*a*) Large forms ($18\mu \times 4\mu$, without the undulating membrane) with broad, rounded posterior portion, and a well-marked undulating membrane, staining pink with Giemsa (Plate III, Figs. 16 and 17). The nucleus is situated in the posterior half of the body; it is rather large, and stains deep rose-red, but is much obscured by granules in the surrounding cytoplasm. The blepharoplast is situated close to the nucleus—slightly anterior or lateral: it is relatively large, and is broadly rod- or crescent-shaped: it takes on a deep purplish-black stain. The undulating membrane arises close to the blepharoplast, but its exact origin is difficult to make out: the free flagellar portion is very short. The cytoplasm stains pink in the neighbourhood of the undulating membrane: near the nucleus

it is crowded with irregular dark-staining red and purple granules. (*b*) Slender forms ($10{\cdot}8\mu \times 2\mu$), with a *Herpetomonas*-like facies, appear here and there (Plate III, Fig. 18). The posterior portion of the body is tapering; the undulating membrane is very narrow, and has a long free flagellar portion (9μ). The nucleus is small, and is situated half-way along the body. In one case a minute, deeply-staining granule could be seen lying between the nucleus and the slightly anterior blepharoplast—otherwise the cytoplasm was singularly free from dark-staining granules. (*c*) Still another type may occasionally be seen (Plate III, Fig. 19 *a*, *b*), of very small size (4μ long), with an oval to cigar-shaped body, a long free flagellum, no undulating membrane discernible, and a relatively large posterior blepharoplast, generally situated behind a vacuole. Of nucleus there is no trace, unless the granules distributed in the cytoplasm be regarded as a nucleus in a diffuse condition.

Only one clearly marked instance of division was met with (Plate III, Fig. 17). The daughter forms were still attached by the distal portion of the undulating membrane, which is apparently the last organ to divide.

(3) *Post-flagellates.* Most of the parasites are in this condition. Their dimensions vary from $2{\cdot}5\mu \times 2{\cdot}5\mu$ to $7\mu \times 6\mu$, and all stages of the gradual rounding-up and encystment can be seen (Plate III, Figs. 21 to 26). A rosette of oblong dividing forms is shown in Fig. 20. The parasites are attached centrally by their reduced flagella, the blepharoplasts have in two cases divided, and the characteristic strings of chromatin-like granules in the cytoplasm are well seen. The distal portion of the flagellum becomes gradually reduced to a sort of pink-staining "brush" which is gradually absorbed. The intracellular portion is distinguishable for some time, and it appears to take its origin in a pinkish, vacuole-like area in front of the nucleus. Its connection with the blepharoplast becomes lost, and that body may wander to the posterior end of the cell, or become indistinguishable from other granules in the cytoplasm (Figs. 22, 23 and 26). The nucleus generally stains rose-red without distinct reticulum, the chromatin being massed peripherally. In Fig. 21 the nucleus is apparently the first part of the cell to divide, and is shown in process of constriction: in Fig. 27 the two halves are redividing before the bulk of the cell has completed the first division. Frequently the nucleus shows signs of disappearing altogether: in Fig. 22 it has fragmented into three parts, and in Figs. 24 and 25, it is reduced to a mass of diffuse chromidia. The most advanced stage of

encystment that I saw is shown in Fig. 26. The cell is rounded off; the cytoplasm and the nucleus have taken on a more compact appearance; the blepharoplast is indistinguishable from other deep-staining bodies in the cell; the periplast stains faintly red, but there is no thick cyst-wall.

Without actual observation of the process of conjugation, it seems rash to draw arbitrary distinctions between so-called "male" and "female" forms. It is evident, even from what I have indicated above, that complicated intracellular readjustments take place during the encysting stages of *Herpetomonas ctenophthalmi* and *Crithidia hystrichopsyllae*, but with such a limited material and no opportunity for close study of the living organism, it were unwise to force an interpretation.

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[1] The complete literature on this subject may be found at the end of an article by Patton and Strickland on p. 343 of Vol. I. of this *Journal*.

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EXPLANATION OF PLATE III.

All figures drawn under oil-immersion $\frac{1}{12}$″ Zeiss, ocular 4. Figs. 2 and 3 are drawn to a scale $\frac{1}{3}$ less than the other figures.

Figs. 1—15. *Herpetomonas ctenophthalmi.*

Fig. 1. Adult flagellate with intact flagellum.
Fig. 2 (*a*, *b* and *c*). Flagellates, showing reduction of flagellum.
Fig. 3. Radiate group of flagellates.
Fig. 4 (*a* and *b*). Forms with diffused chromatin: (*b*) shows unequal division.
Figs. 5–11. Stages of encystation.
Fig. 12. Degeneration of nucleus (?).
Fig. 13. Formation of chromidia.
Fig. 14 (*a*). Cyst with dividing nucleus; (*b*) cyst with no apparent nucleus.
Fig. 15. Small flagellate without nucleus.

Figs. 16–27. *Crithidia hystrichopsyllae.*

Fig. 16. Large flagellate.
Fig. 17. Flagellates in last stage of division.
Fig. 18. Slender flagellate.
Fig. 19 (*a* and *b*). Small flagellates with no apparent nucleus.
Fig. 20. Group of encysting forms in process of division.
Figs. 21–26. Stages of encystation: in Fig. 21 the nucleus is dividing: in Fig. 25 the flagellum is doubled.
Fig. 27. Large cyst in division.

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NATURAL HISTORY

THE REGIONAL DISTRIBUTION OF FLEAS ON RODENTS.

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THIS investigation was prompted by the fact that in the study of the lesions of natural plague infection among rats in San Francisco it was found that the anatomical changes produced by the disease were practically identical with those described by the British Plague Commission[1] in India, except that in San Francisco the location of the bubo in the great majority of cases was in the inguinal region, and practically none of the buboes were found in the cervical region. In India, however, the great majority of the buboes, 74·3 % in 2956 rats, were located in the cervical region, a comparatively small percentage, 14·8 %, were in the inguinal region and 10·9 % in the axilla. These figures refer to single buboes only. It was assumed that fleas were responsible for the epizootic among the rats here, as had been shown to be the case in India.

Wherry, Walker and Howell[2], in a paper on rat plague, as observed in San Francisco, state that the buboes they observed were distributed as follows: one in the cervical region, one in the axilla, and six in the inguinal region. One of the present writers[3] reported that in San Francisco, among 29 rats showing single buboes, no case of cervical bubo

[1] *Journal of Hygiene*, Vol. VII. No. 3, p. 386.

[2] *Journal American Medical Association*, April 11, 1908, Vol. L. No. 15.

[3] *Public Health Reports*, Washington, Vol. XXXIII. No. 30, p. 1051.

had come under observation, while in 21 cases (72·4 %) the lesion was in the groin, in six cases (20·4 %) in the axilla and twice (6·9 %) in the location of the pelvic glands. In the later experience in San Francisco no case of cervical bubo has been observed.

The Indian Plague Commission[1] found that the situation of the buboes in nearly 200 *guinea-pigs* experimentally infected with plague by fleas was as follows: 88·9 % were found in the neck, 9·3 % in the groin and 1·8 % in the axilla. These figures are given for those animals having single buboes, constituting 90·5 % of all. The remainder had multiple buboes, *i.e.* in two or more situations, and in these animals the neck glands were affected in every instance. An effort was made by these observers to ascertain the connection between the situation of plague buboes and the distribution of the fleas on the body of the animal. It had been noted as a common observation that the favourite situation of fleas on the body of animals was the under surface of the neck and beneath the chin, *i.e.* in the cervical region. An actual count was made on the bodies of 53 live guinea-pigs which were placed singly in a wide-mouthed, stoppered bottle containing a piece of wool soaked in chloroform, thus stupefying both the animal and the fleas at the same time. The fleas were removed and a careful record taken of the situation in which they were found. A census of the anaesthetized parasites showed 65·3 % taken on the head and neck, 11·5 % on the forelegs and axillae, 12·9 % on hind legs and groin, and 10·2 % on the trunk of the animal. The commonest situation in which fleas occur on guinea-pigs was thus demonstrated to be the head and neck, the region drained by the cervical lymph glands. In these experiments and observations the species of flea concerned was that common to the rats of India, *Loemopsylla cheopis*, Rothschild.

The only definite statement we have encountered on the subject of the regional distribution of fleas on *rats* is that of Pound[2], who, in speaking of the distribution of fleas on their host, says, "Thus with regard to rats, fleas more commonly occur, when infesting these rodents, about the head and neck than elsewhere."

The Indian Plague Commission makes no statement, so far as we have been able to determine, as to the regional distribution of fleas on *rats*, but the fact that they found the majority of the buboes in the cervical region leads one to draw the inference that this is the region most infested by fleas in India. As cited above, they demonstrated by

[1] *Journal of Hygiene*, Vol. VI. No. 4, p. 465.

[2] *Report on Plague in Queensland*, 1900–1907, p. 143, B. Burnett Ham.

actual count that on *guinea-pigs* the head and neck is the part of the body on which the majority of fleas are found. As will be seen from our tables we have been unable to verify this observation.

It would not be without precedent to find that different species of the same family of insects infest different parts of the body. A very definite regional distribution of certain ectoparasites of man, namely, the lice, has long been recognized. Thus we find *Pediculus capitis* on the head, *Pediculus vestimenti* on the general body surface, and *Phthirius inguinalis* on the pubic region.

In San Francisco it was not practicable to conduct actual flea transmission experiments of plague with either rats or guinea-pigs. In the absence of such experiments it seemed to us that perhaps some clue as to the difference in the location of plague buboes in rats here and in India might be obtained by a careful enumeration of the fleas taken from the various regions of the body of the rats. The work was later extended to include guinea-pigs and squirrels. It should be stated that this work was done after the subsidence of the natural plague among rats in San Francisco and vicinity. The rats have been carefully combed by ourselves or under our immediate supervision, and the figures clearly show that the hind part of the body yields a much larger number of certain species of fleas than is to be obtained from the head and neck or from the forequarters, while another species is confined almost exclusively to the head and neck. We may state that it is the general impression among the attendants who have combed rats here in the routine flea enumeration work, that the majority of the fleas come from the hind regions of the body.

We have combed a number of *squirrels*, carefully observing the regions from which the fleas were obtained. It will be seen that here again the common squirrel flea (*Ceratophyllus acutus*) prefers the hind region of the body, and *Hoplopsyllus anomalus*, while taken from but one squirrel, showed a marked preference for the hind quarters.

Without insisting upon the significance of these findings we would call attention to the fact that of the four naturally infected plague squirrels reported by Acting Assistant Surgeon Wherry[1], Public Health and Marine Hospital Service, three had buboes in the inguinal region, and one of these had in addition a bubo in the axilla. The fourth squirrel had no bubo. There was no case of cervical bubo.

A number of fleas have been placed on *guinea-pigs* and after allowing sufficient time for the insects to orient themselves (from one to five days)

[1] *Journal of Infectious Diseases*, Vol. v. No. 5.

the animals have been chloroformed and the parasites removed. In the case of *C. fasciatus* and *L. cheopis* the fleas were generally placed on the trunk or on the back of the neck; in the case of *Ct. musculi* they were placed on the hind part of the body. In other words, we have placed the fleas in such positions upon the animal that, judging from our experience with rats, it would be necessary for the fleas to move about in order to reach the positions of election for each species.

We have used special care in the guinea-pig experiments as we wished the results to be comparable as far as possible with those of the English workers in India. When we wished to remove the fleas the guinea-pigs were wrapped in a towel saturated with ether, thus stupefying the parasites and preventing any alteration of their position.

In the case of rats the determinations have been made for the most part with animals brought to the laboratory alive, and the fleas removed by one of the procedures to be mentioned later. In a few cases we have put fleas on a rat previously freed of fleas and confined in a mouse jar. On the following day the animal was combed and a note made of the region from which the parasites came. In other cases we have transferred fleas from rats to squirrels and *vice versâ,* and have noted the distribution of the parasites on the new host.

In some cases the rat was injected intraperitoneally with a solution of potassium cyanide. This invariably killed the animal within a minute, and the search for parasites was made at once. This method was intended to prevent any change of position of the fleas due to such struggling as occurred when the animal was coming under the influence of the anaesthetic. Occasionally the animal, as soon as it was dead, was wrapped in filter paper saturated with chloroform; this effectually prevented any change of position on the part of the parasites. The results obtained in this way did not differ materially from those obtained by the simpler process of chloroforming the rat and the fleas simultaneously, and as the latter method was more convenient we have employed it in the majority of the determinations.

The results obtained by the various methods with each of the species of mammals and of fleas were essentially the same: *i.e.* the method employed had no influence on the result of the enumeration. Some of these regional determinations were made in Oakland, California, through the courtesy of Passed Assistant Surgeon Carroll Fox.

The average number of fleas on a single rodent was generally so small that, for the purpose of eliminating errors, we have grouped together a number of rodents, including in one group all combed on the same day.

No. in Group	Host	Head and Neck						Fore Quarters						Hind Quarters						Total
		Fasc.		*Cheop.*		*Mus.*		*Fasc.*		*Cheop.*		*Mus.*		*Fasc.*		*Cheop.*		*Mus.*		
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
13	*Mus norvegicus*	—	—	—	—	2	1	—	—	—	—	—	—	4	12	—	1	—	—	20
6	,,	1	1	—	—	1	2	—	1	—	—	—	—	—	8	3	1	—	—	18
9	,,	—	2	—	—	2	3	1	2	—	—	1	3	4	9	—	2	—	—	29
8	,,	—	—	1	1	2	2	3	2	—	1	—	—	2	12	11	10	—	—	47
8	,,	—	1	—	—	—	—	—	1	—	—	—	—	9	10	—	1	—	—	22
8	,,	—	—	—	—	1	3	2	1	—	—	—	2	—	11	2	1	—	—	23
9	,,	1	4	1	3	1	17	—	1	—	—	—	—	7	23	2	4	—	—	64
10	,,	—	—	2	6	—	7	—	—	2	4	—	—	5	5	10	16	—	1	58
6	,,	—	—	—	1	2	10	—	—	—	1	—	2	—	5	6	14	—	—	41
10	,,	—	—	—	—	—	—	—	—	—	—	—	—	3	6	—	6	—	—	15
1	,,	—	—	—	—	4	18	—	—	—	—	—	—	—	—	—	—	—	—	22
18	,,	—	—	—	—	—	—	—	—	—	1	—	1	2	2	4	9	—	—	19
14	,,	—	—	—	—	1	—	3	5	—	3	—	—	10	12	1	12	—	—	47
19	,,	—	—	—	1	—	2	—	1	—	1	—	—	—	6	8	18	—	—	37
18	,,	—	—	—	—	—	—	2	2	—	2	—	—	2	8	17	26	—	—	59
19	,,	—	—	—	—	1	—	3	7	—	—	—	—	35	43	—	—	—	—	89
5	*Mus rattus*	—	1	—	—	—	6	—	—	—	—	—	1	3	10	—	—	—	—	21
181		2	9	4	12	17	71	14	23	2	13	1	9	86	182	64	121	—	1	
Total both sexes		11		16		88		37		15		10		268		185		1		631

Rats killed by the intraperitoneal injection of potassium cyanide.

No. in Group	Host	Head and Neck						Fore Quarters						Hind Quarters						Total
		Fasc.		*Cheop.*		*Mus.*		*Fasc.*		*Cheop.*		*Mus.*		*Fasc.*		*Cheop.*		*Mus.*		
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
6	*Mus norvegicus*	—	—	—	—	2	3	—	—	—	—	—	1	1	4	—	—	—	—	11
15	,,	—	—	1	—	3	6	—	1	—	1	1	1	4	13	11	8	—	—	50
7	,,	—	—	—	—	7	21	—	—	—	—	1	6	1	4	1	1	—	—	42
9	,,	—	—	—	—	3	11	—	—	1	4	—	1	1	6	5	8	—	—	40
37	,,	—	—	1	—	15	41	—	1	1	5	2	9	7	27	17	17	—	—	
Total both sexes				1		56		1		6		11		34		34				143

Fleas placed on rats confined in mouse jar.

No. in Group	Host	Head and Neck						Fore Quarters						Hind Quarters						Total
		Fasc.		*Cheop.*		*Mus.*		*Fasc.*		*Cheop.*		*Mus.*		*Fasc.*		*Cheop.*		*Mus.*		
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
12	*Mus norvegicus*	—	1	3	4	3	3	—	—	—	—	—	—	1	8	13	33	—	—	69
Total both sexes		1		7		6								9		46				69

For the purpose of these determinations that part of the animal lying in front of the fore legs was called "head and neck." The "hind quarters" included everything posterior to the middle of the trunk, and the parts lying between this and the "head and neck" was called the "fore quarters."

A summary of the three tables, including a total of 230 rats, shows the percentages of species of fleas as follows:

	Fasciatus	*Cheopis*	*Musculi*
Head and Neck	3·3 %	7·7 %	87·2 %
Fore Quarters	10·5	6·8	12·2
Hind Quarters	86·2	85·5	·6

Distribution of squirrel fleas (Ceratophyllus acutus) on rats.

No. in Group	Host	Fore Quarters *C. acutus*		Hind Quarters *C. acutus*		Total
		M	F	M	F	
9	*Mus norvegicus*	1	1	18	16	38
Total both sexes		2		34		
Percentage		6		94		

Distribution of squirrel fleas (Ceratophyllus acutus and Hoplopsyllus anomalus) on squirrels (Citellus beecheyi).

No. in Group	Host	Head and Neck				Fore Quarters				Hind Quarters				Total
		C. acutus		*Anomalus*		*C. acutus*		*Anomalus*		*C. acutus*		*Anomalus*		
		M	F	M	F	M	F	M	F	M	F	M	F	
11	*C. beecheyi*	19	16	—	—	38	58	—	—	93	118	—	—	342
8	,,	5	—	—	—	37	44	2	—	42	31	—	—	161
6	,,	14	16	—	—	15	26	—	4	33	39	8	23	178
25		33	37	—	—	90	128	2	4	168	188	8	23	
Total both sexes		70				218		6		356		31		681

From this we find that the percentages are as follows:

	C. acutus	*H. anomalus*
Head and Neck	10·9 %	00·0 %
Fore Quarters	33·8	16·2
Hind Quarters	55·3	83·8

Distribution of rat fleas on guinea-pigs.

	Head and Neck						Fore Quarters						Hind Quarters						
	Fasc.		*Cheop.*		*Mus.*		*Fasc.*		*Cheop.*		*Mus.*		*Fasc.*		*Cheop.*		*Mus.*		
No. of guinea-pigs in group	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	Total
Group 1. *Ceratophyllus fasciatus.*																			
9	1	2	—	—	—	—	2	6	—	—	—	—	11	37	—	—	—	—	—
Total both sexes	3						8						48						59
Group II. *Loemopsylla cheopis.*																			
9	—	—	2	1	—	—	—	—	3	6	—	—	—	—	10	33	—	—	—
Total both sexes			3						9						43				55
Group 3. *Ceratophyllus fasciatus, Loemopsylla cheopis and Ctenopsyllus musculi.*																			
10	1	2	1	3	—	10	10	13	—	3	—	4	13	45	2	10	—	—	—
Total both sexes	3		4		10		23		3		4		58		12				117
Summary of three groups.																			
No. g.-pigs combed 28	2	4	3	4	—	10	12	19	3	9	—	4	24	82	12	43	—	—	—
Total both sexes	6		7		10		31		12		4		106		55				231

These figures yield the following percentages:

	C. fasciatus	*L. cheopis*	*Ct. musculi*
Head and Neck	4 %	10 %	72 %
Fore Quarters	22	16	28
Hind Quarters	74	74	0

Summary and Conclusion.

It would appear from the data presented that the favourite location for the common rat fleas of this vicinity, *Ceratophyllus fasciatus*, Bosc., and *Loemopsylla cheopis*, Roth., is about the hind quarters of the rat, while *Ctenopsyllus musculi* (Dugès), Wagner, prefers the region of the head and neck.

The same regional distribution of rat fleas was found in the case of the guinea-pigs.

Squirrel fleas are most numerous on the hind quarters.

These observations, while they are not to be insisted upon as throwing any special light upon the regional distribution of buboes in naturally infected plague rats, are of particular interest in showing the very constant preference of *Ct. musculi* for the region of the head and neck.

In regard to the other species of fleas it should be borne in mind that the skin areas are not of equal dimensions; that of the head and neck being smallest; that of the hind quarters being the largest; and the skin area of the fore quarters being between these in size.

ON THE SEASONAL PREVALENCE OF *TRYPANOSOMA LEWISI* IN *MUS RATTUS* AND IN *MUS DECUMANUS* AND ITS RELATION TO THE MECHANISM OF TRANSMISSION OF THE INFECTION.

By G. F. PETRIE, M.D., and C. R. AVARI.

No precise observations so far as we are aware have been published hitherto regarding the seasonal prevalence of any of the infections in man or animals—malaria excepted—which are definitely associated with the presence in the blood of the host of a protozoan. For this reason we have thought it desirable to record an extensive series of observations on the occurrence of *T. lewisi* in *M. rattus* and *M. decumanus* in Bombay made during a period of 14 months in the years 1905—1906.

A recent paper by Nuttall (1908) on the transmission of *T. lewisi* by means of insects has revived interest in the question of the mode of transmission of this parasite from rat to rat. We believe that our observations have a bearing upon this problem and we shall therefore bring under review in due course the epidemiological factors which must be reckoned with in a discussion of the problem.

While reserving full discussion of these factors until later it may be stated at once that evidence will be brought forward which points to a developmental cycle taking place in the insects transmitting the infection. This evidence, derived as it is from purely epidemiological sources, is perhaps of especial interest in view of Kleine's (1909) important experiments on the transmission of *T. brucei* confirmed recently for *T. gambiense* by Bruce (1909)—experiments which make it practically certain that these trypanosomes undergo a cycle of development in the *Glossina palpalis.* Moreover our observations seem to us to shed some fresh light on the transmission of *T. lewisi* and probably also of all insect transmitted trypanosomes in that they give a clue to the dominant factor influencing the cycle of development.

It is somewhat curious perhaps that notwithstanding the large amount of work devoted to the biology of *T. lewisi*—work which has aided materially in guiding researches upon the pathogenic trypanosomes—very scanty attention appears to have been paid to the question of possible seasonal variations in the prevalence of the infection amongst rats. Before bringing forward our own observations we may briefly cite references to the subject by other writers.

It is interesting to note that Vandyke Carter (1887) who in 1885 first observed the occurrence of *T. lewisi* in Bombay rats (chiefly *M. decumanus* and *M. rattus* (?)) clearly recognised the possibility of a seasonal prevalence of the infection. Thus Carter states in his memoir that from his observations—referring presumably to their paucity—"valid inference regarding a possible seasonal prevalence could not be made." Again he writes: "In the same connection I would here allude to the evident narrow place-limitation or endemicity of both rat infection and the 'surra' disease with probably also a distinct seasonal variation of prevalence."

Lingard (1895) examined a large number of rats (*M. decumanus*) in Bombay during several years and is quoted by Laveran and Mesnil in their book on trypanosomes as having found that during the rainy season, June to October, 42 % of the rats were infected with trypanosomes, while during the dry season, November to May, only 28 % were infected. These observations of Lingard's will be referred to at greater length below.

Musgrave and Clegg (1903) remark that in Manila rats (thousands examined) *T. lewisi* has been found in from 20—65 % of the individuals examined, varying according to the season and to the locality from which they were received. Unfortunately these authors content themselves with this bare statement—details are absolutely lacking.

Swingle (1907) recites a few facts from his own experience in Nebraska which seem to indicate a seasonal prevalence of the infection. "Of the 17 one-fourth grown rats examined in the autumn and winter not one was infected with either lice, fleas or trypanosomes, while of the seven caught the following spring at the same place all had fleas and four were harbouring the parasite."

Lastly, Yakimoff (1907) is cited by Mesnil as stating that in an investigation of the distribution of the infection amongst the rats in St Petersburg the proportion of rats infected was greater in the warm than in the cold season.

The present series of observations was begun on 1st September, 1905,

and was continued without intermission until 19th October, 1906, a period of nearly 14 months[1]. The rats were brought from all parts of Bombay city and island for the purposes of the Plague Commission then working at the laboratory at Parel. The observations were made generally on five or six days of each week. Only "young" rats were examined for the presence of trypanosomes, i.e. in accordance with the arbitrary standard adopted by the Commission, those weighing 70 and 100 grammes or less in the case of *M. rattus* and *M. decumanus* respectively; no rats below these weights were found pregnant. Young rats were selected with the idea of eliminating the factor of immunity acquired in adult rats either by their having harboured the infection at some previous time or by vaccination with doses of infected blood insufficient to bring about an infection. When brought to the laboratory the young "live" rats were killed and at once dissected. Blood was pipetted from the heart and spread in a somewhat thick layer upon clean slides. The blood films after fixation with absolute alcohol were stained with carbol-thionin and examined under an oil immersion lens. It was found that carbol-thionin had the advantage as a diagnostic stain for trypanosomes of staining the red blood corpuscles very feebly so that a film of moderate thickness could be searched for the parasites without difficulty.

In all, 1832 *M. decumanus* and 2651 *M. rattus*, a total of 4483 rats, were examined during the period of investigation. The results are shown in the tables (I, II and III) and in Chart I. It will be noted (Tables I, II and III) that, as might be expected, there is no marked difference in the incidence of the infection on males and females of both species. Table III also shows that the percentage of *M. rattus* infected during the whole period does not differ markedly from the percentage of infected *M. decumanus*.

It is interesting to compare these results with those obtained by previous observers in Bombay. Vandyke Carter in 1885 examined 210 rats for trypanosomes and found 25 infected, i.e. nearly 12 %. From November 1890, to May 1895, Lingard in the course of his work on

[1] We may note that the primary object we had in view in undertaking the investigation was in order to determine if possible, a seasonal prevalence of trypanosomes in rats which might give a clue to the season of rat-flea prevalence, an important question in plague epidemiology. In this we were influenced by the experiments of Rabinowitsch and Kempner (1899) which seemed to warrant the belief that rat fleas could act as transmitters of the infection in nature. As it happened the Plague Commission were able to obtain direct evidence during the year subsequent to our investigations as to a season of rat flea prevalence in Bombay.

TABLE I.

Giving results of examination of M. rattus *for trypanosomes.*

Monthly periods	Males			Females			Total *		
	Examined	Infected	Percentage	Examined	Infected	Percentage	Examined	Infected	Percentage
September 1905	97	38	39·2	117	41	35·0	302	106	35·0
October	137	71	51·8	114	61	53·5	251	132	52·6
November	101	48	47·5	80	44	55·0	191	94	49·2
December	121	58	47·9	118	54	45·8	239	112	46·9
January 1906	116	55	47·4	131	42	32·1	247	98	39·7
February	82	29	35·4	93	21	22·6	175	50	28·6
March	69	10	14·5	78	14	17·9	147	24	16·3
April	68	16	23·5	69	13	18·8	137	28	20·4
May	42	11	26·2	50	16	32·0	92	27	29·3
June	118	59	50·0	89	55	61·8	207	114	55·0
July	93	58	62·4	81	44	54·3	174	102	58·6
August	84	51	60·7	112	74	66·0	196	125	63·8
September	73	45	61·6	84	48	57·1	157	93	59·2
October	72	43	59·7	64	40	62·5	136	83	61·0

* These columns include a number of rats in which the sex was not noted.

TABLE II.

Giving results of examination of M. decumanus *for trypanosomes.*

Monthly periods	Males			Females			Total *		
	Examined	Infected	Percentage	Examined	Infected	Percentage	Examined	Infected	Percentage
September 1905	59	32	54·2	66	29	43·9	196	70	35·7
October	128	43	33·6	101	33	32·6	229	76	33·2
November	81	37	45·7	72	44	61·1	183	87	47·5
December	94	49	52·1	94	44	44·4	208	93	44·7
January 1906	85	39	45·9	110	46	41·8	219	85	38·8
February	49	7	14·3	63	17	27·0	112	24	21·4
March	52	6	11·5	43	14	32·5	95	20	21·0
April	35	2	5·7	39	3	7·7	77	5	6·5
May	8	1	12·5	10	2	20·0	18	3	16·6
June	55	29	52·7	47	22	46·8	102	51	50·0
July	61	25	41·0	52	32	61·5	113	57	50·4
August	52	26	50·0	60	35	58·3	112	61	54·5
September	40	22	55·0	34	16	47·0	74	38	51·4
October	47	28	59·5	47	26	55·3	94	54	57·4

* These columns include a number of rats in which the sex was not noted.

TABLE III.

Summarising results of examination of M. decumanus *and* M. rattus *for trypanosomes.*

M. decumanus			*M. rattus*		
Males examined	= 846	40·9 %	Males examined	= 1273	46·5 %
Males infected	= 346		Males infected	= 592	
Females examined	= 838	43·3 %	Females examined	= 1280	44·3 %
Females infected	= 363		Females infected	= 567	
Total* examined	= 1832	39·5 %	Total* examined	= 2651	44·8 %
Total infected	= 724		Total infected	= 1188	

* Includes a number of rats in which the sex was not noted.

Surra examined 3105 *M. decumanus* and found 35 % infected. Our observations show that in 1905—1906, 40—45 % of the Bombay rats were infected. These results taken as a whole appear to confirm the conclusion arrived at by Laveran and Mesnil on experimental grounds in their work on *T. lewisi* that "immunization through the placenta or by lactation is exceptional if it occurs at all." At least the figures give no ground for belief that during a period of 20 years the rat population in Bombay has become less susceptible as the result of a cumulative hereditary transmission of immunity against the infection.

Coming now to the consideration of the question of the seasonal prevalence of the infection, it is clear from the details given in the tables and represented graphically on the chart that a marked seasonal variation in prevalence exists, and that the curves of prevalence for *M. rattus* and *M. decumanus* present a noteworthy resemblance. Thus in the months June to December the percentage figures of infected animals of both species rise above the mean for the year, while in the remaining months the percentage figures fall below the mean. The lowest percentages of infected animals are found in March and April, corresponding to 6·5 % infected *M. decumanus* and 16·3 % infected *M. rattus*, while the highest percentages appear from the chart to correspond to the month of August, in which 54·5 % *M. decumanus* and 63·8 % *M. rattus* proved to be infected.

During the preparation of this paper for publication we have obtained a striking confirmation of these results while consulting Lingard's original report (1895) of his observations on *T. lewisi* in Bombay. Lingard in this report (*Summary of Further Report on Surra*, Bombay 1895) gives the data which led him to the conclusion that a

larger percentage of rats are infected during the months June to October than during the remaining months of the year. His percentage figures of infected animals (*M. decumanus*) for each month from May 1894, to April 1895 are reproduced in Table IV; the total number of animals examined during this year was 2102. We have

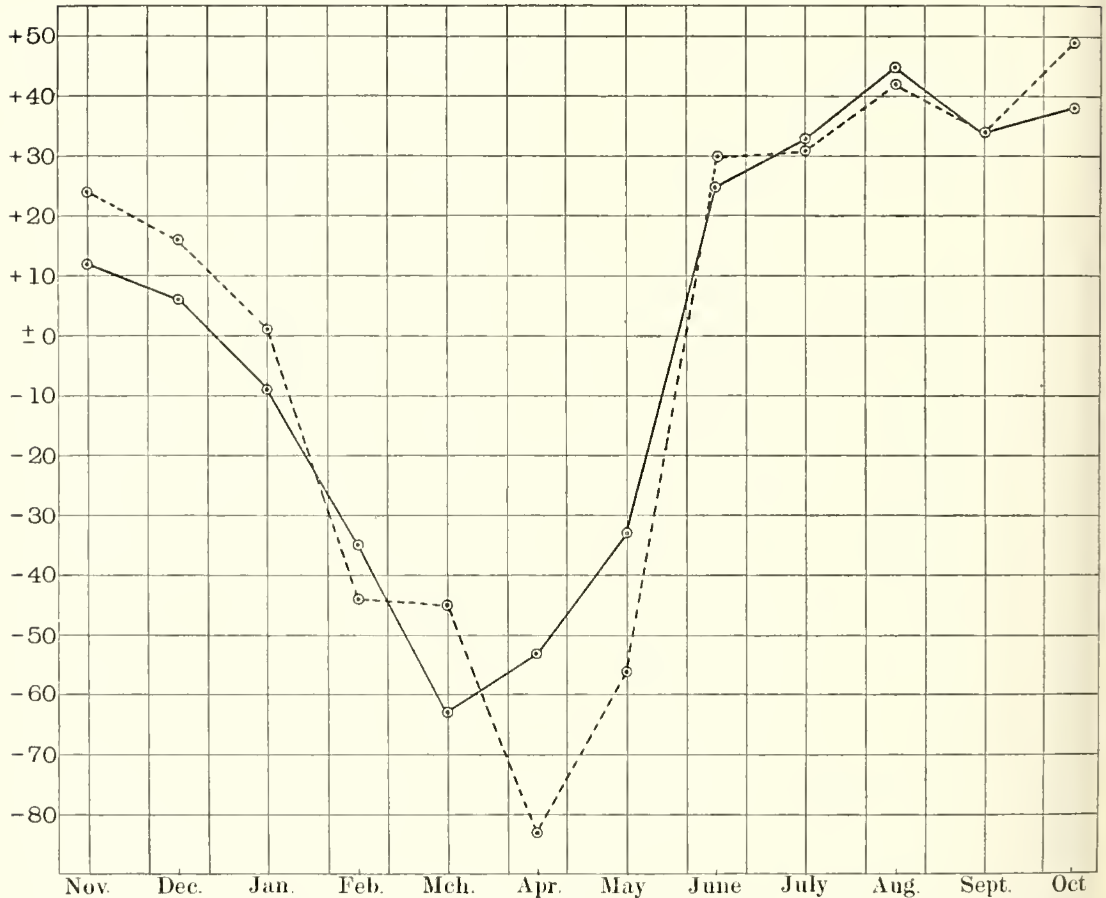

Chart I. Prevalence of trypanosomes in *Mus rattus* and *Mus decumanus*, Bombay, 1905, 1906.

——= *Mus rattus*.

-----= *Mus decumanus*.

represented these figures in Chart II in the same manner as has been done in Chart I, and it will be seen that the prevalence curve based on Lingard's figures approximates very nearly to our own curves[1].

[1] For convenience of comparison the months in Chart II are arranged in an order which is not entirely consecutive, but this does not appear to us to invalidate a general comparison between the two charts.

Lingard apparently examined all rats available without reference to age.

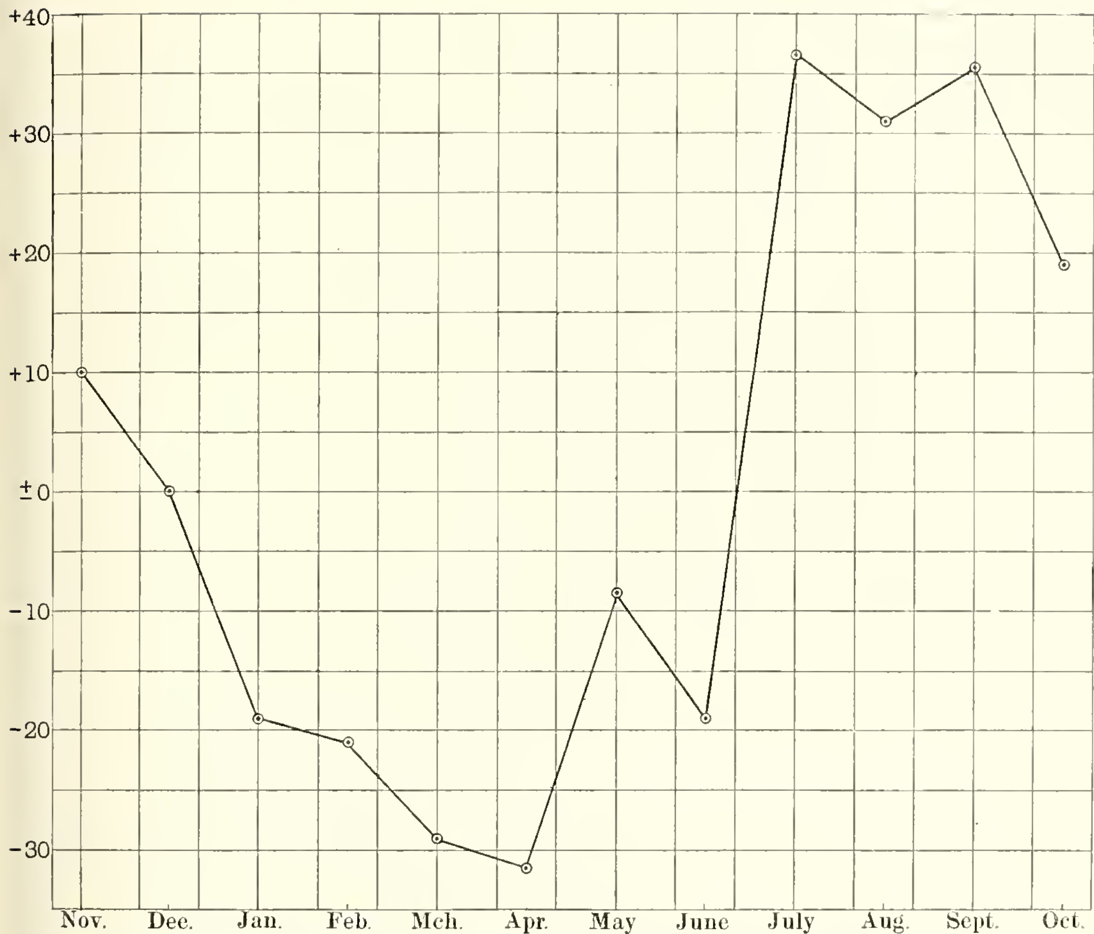

Chart II. Showing prevalence of trypanosomes in *Mus decumanus*, Bombay, 1894, 1895. (Constructed from Lingard's figures.)

With regard to the interpretation of these curves we cannot but believe that this is bound up with the mechanism of transmission of the infection. The present state of knowledge on this subject does not justify us in bringing forward any positive conclusions in the matter. Nevertheless some considerations may be advanced here which in our view assist in explaining the facts narrated above.

If it be admitted that the explanation of the variations in prevalence is probably to be found in the mode of transmission of the parasite, two hypotheses suggest themselves as affording a reasonable interpretation of the facts. The first hypothesis is that the trypanosome prevalence is associated with a seasonal prevalence of the insects transmitting the infection, and the second possibility is that apart from insect prevalence the conditions for successful transmission of the infection by insects vary at different seasons of the year.

TABLE IV.

Showing prevalence of trypanosome infections in M. decumanus (*abstracted from Lingard's Reports*) *and of Adie's* Leucocytozoon *in* M. rattus (*abstracted from Plague Commission's Reports*).

Percentage of *M. decumanus* infected with trypanosomes		Percentage of *M. rattus* infected with leucocytozoon	
May 1894	31·2	December 1905 ...	16·8
June	27·5	January 1906 ...	14·6
July	46·7	February	7·1
August	43·0	March	4·1
September	46·3	April	0·7
October	40·4	May	5·4
November	37·5	June	22·4
December	34·1	July	21·7
January 1895 ...	27·5	August	21·6
February	26·6	September	19·2
March	24·1	October	16·7
April	23·4	November	15·8

The question of the correlation of the prevalence of the infection with the seasonal prevalence of the ectoparasites of rats.

The insects which infest the Bombay rats are fleas (*Loemopsylla cheopis*, Rothsch.) and lice. With regard to the prevalence of rat fleas in Bombay the extensive data collected and published by the Plague Research Commission (1908) are happily available for comparison. It seems scarcely necessary to point out that rat fleas are very abundant on the rats in Bombay. The Commission found that the average number of fleas per rat taken from *M. rattus* during a year's investigation was 3·7, while the average number per rat in *M. decumanus* was 8·5. They further determined that there is a definite seasonal prevalence of rat fleas in Bombay. The curves of rat flea prevalence are reproduced from the Commission's reports in Chart III, from which it will be seen that the highest point of the curve of fleas taken from *M. rattus* falls in March and April and corresponds to an average per rat for these months of 5·2 fleas, while the lowest point is found in November, corresponding with an average of 2·5 fleas per rat. The highest point in the *M. decumanus* curve corresponds to an average of 14 fleas per rat in April and the lowest point to an average of 4·2 per rat in September. When a comparison is instituted between the rat flea curves of prevalence and the curves representing the prevalence of trypanosomes it is at once obvious that

the curves in the two charts show a completely inverse relation—the off season in the one case corresponding to the season of prevalence in the other.

Before attempting to compare these charts from the point of view of a possible causal relationship, it is necessary to take into account the mean interval elapsing between the date of infection of the rats and the date on which examination revealed the parasites in their blood. It is impossible to determine this interval with any degree of accuracy, but it may be pointed out that all the rats examined were young, probably

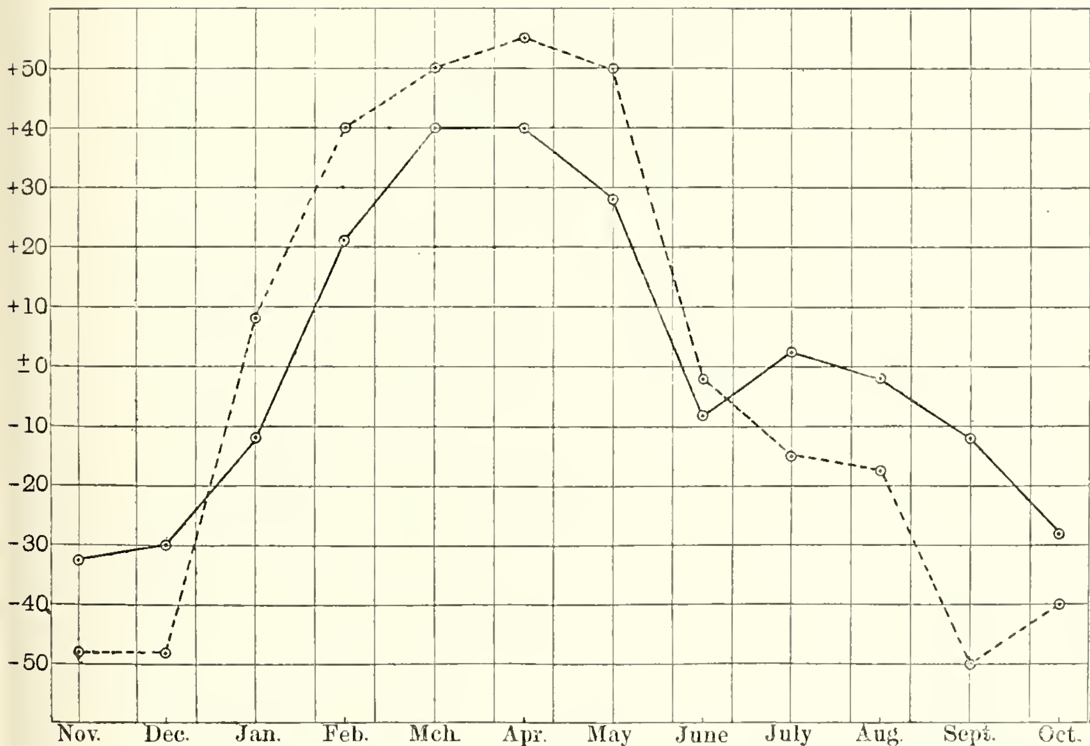

Chart III. Showing Rat flea prevalence in Bombay, 1906, 1907. (From the Plague Commission's Reports, *Journ. Hygiene*, Vol. VIII.)

——— = Fleas on *Mus rattus*.

- - - - - = Fleas on *Mus decumanus*.

on an average 4—6 weeks old. It may be noted also in this connection that Laveran and Mesnil (1901) have found experimentally that in young rats (30—100 grammes) the incubation period, i.e. the interval between inoculation of infected blood and the appearance of the parasites in the circulation, is very short—usually only 24 hours. For these reasons we think that a general comparison may justly be made between

the two charts. Must it be supposed then, as at first sight it appears, that there is no relation between the prevalence of rat fleas in Bombay and the occurrence of trypanosomes in the rats? It cannot be doubted that if rat fleas can act as transmitters of *T. lewisi* the propagation of the infection amongst the rats must be aided by a seasonal increase in the number of rat fleas. The Plague Commission, for example, have shown that the rat flea prevalence in Bombay is associated in a marked manner with the prevalence of plague amongst the rats. It is difficult, in view of Nuttall's (1908) recent transmission experiments with rat fleas (*Ceratophyllus fasciatus* and *Ctenophthalmus agyrtes*), to suppose that *Loemopsylla cheopis* is incapable of conveying the infection. Moreover certain observations of our own in Bombay strongly suggest that the latter insect is able to originate an outbreak of the infection. A batch of tame white rats sent out from England were confined together in a cage which was deposited on the floor of a room in the laboratory; this room was over-run with *M. rattus* and *M. decumanus*, and needless to say was infested with rat fleas. After a brief interval a number of the English rats died and trypanosomes were found in their blood; a few rat fleas were captured on some of these rats. Now the construction of the cage was such that rat fleas on the floor of the room might readily gain entrance into the cage, whereas transference of lice from the wild to the tame rats did not seem at all likely.

There is one point which deserves attention in a discussion of the relation between the prevalence of trypanosomes and of rat fleas. As the Plague Commission remark in their report, fleas are most abundantly found in the haunts of their hosts owing to the fact that the houses or nests of the host are *par excellence* the breeding places of these insects. It may be therefore that owing to the "concentration" of fleas in the nests and burrows of the rats the opportunities for infection of young rats tend as it were to be levelled up. This explanation is not, however, wholly adequate, and there remains therefore the supposition that during the season of maximum prevalence of trypanosome infections other factors in the successful transmission of the infection are operative in spite of a diminution in numbers of the transmitting insects and that similarly during the off season of trypanosome infections these factors are partially in abeyance, so that the influence of an increase in the numbers of the transmitting insects is neutralised.

In connection with the question of the infection of young rats in their nests the figures given in the accompanying table (V) were worked out with the idea that they might establish a relation between the age

as judged by the weight of the rats and the degree of infection in the blood. It appears, however, that the difference in weight of the various lots of rats taken is not sufficiently marked to justify any conclusion on this point.

TABLE V.

Comparing the degree of infection with the average weight of M. rattus *and* M. decumanus *infected with trypanosomes.*

Mus rattus.			*Mus decumanus.*		
Degree of infection	Total rats examined	Average weight of rats examined	Degree of infection	Total rats examined	Average weight of rats examined
+	107	47·6 grammes	+	80	62·9 grammes
+ +	86	59·1 ,,	+ +	68	63·8
+ + +	134	58·0 ,,	+ + +	81	58·4

Note :— + = few, + + = fairly numerous, + + + = numerous or very numerous.

No systematic observations have yet been carried out on the prevalence of rat lice in Bombay. In view of Nuttall's (1908) successful transmission experiments with rat lice such observations would be of interest. From general observations, however, it is fairly certain that the season of prevalence of various insect parasites (human-, rat-, cat-fleas and bugs) is the same for all, so that it is probable that the rat flea prevalence curves provide a fair index of the prevalence of rat lice.

Reference may be made here to the relative importance from an epidemiological standpoint of the rat flea and the rat louse in the transmission of *T. lewisi*, assuming that both are capable of transferring the infection from rat to rat in nature. There would seem to be little doubt that the habits of these insects in relation to their host are important in this connection. Thus lice are well known to live constantly upon their host, and this habit must necessarily limit the opportunities for conveyance of infection from rat to rat, although it is readily conceivable that young rats while in the nest, and especially while suckling, might pick up infected lice. Rat fleas on the other hand, from the fact that they spend much of their existence apart from their host, are more likely to convey the infection from rat to rat over considerable distances.

Interpretation of the trypanosome prevalence curves on the view that the conditions for successful transmission vary at different seasons of the year.

It is conceivable that the climatic conditions at a particular season of the year are especially favourable in respect of the persistence or

development of the trypanosomes in the transmitting insect, thereby increasing the chances of successful transmission to the vertebrate host. It is extremely likely for example that if the trypanosomes undergo a developmental cycle in the flea or louse there is an optimum temperature at which such development proceeds. It will be remembered that Grassi (1906) and others have shown experimentally that there is an optimum temperature (77°—86° F.) for sporogony of the malarial parasite in the mosquito, and that Ruge (1906) has given similar proof of the influence of temperature in the case of the *Proteosoma* of birds. Up to the present time, however, developmental stages of *T. lewisi* have not been described in the rat flea, and the observations of Prowazek (1905) on the development of *T. lewisi* in the rat louse have not been accepted by other workers as established.

In Chart IV the temperature curve[1] for Bombay is presented for the year under review together with the trypanosome curve for *M. rattus*[2]; the mean monthly temperature is 79° F.

It will be noticed that the trypanosome curve closely follows the temperature curve. This relationship is indeed so marked as in our view strongly to suggest that temperature is an important factor in determining the prevalence of trypanosome infections in rats. Moreover, since temperature can conceivably exercise an influence only by its effect upon the development of the trypanosomes in the transmitting insects, the relationship between temperature and trypanosome prevalence must be regarded as explicable on this theory. As to the exact significance of the interval separating the two curves it would be premature at present to speculate.

There is yet another influence, however, which climatic conditions may have upon the life history of *T. lewisi* in the agents of transmission. Judging from present knowledge of the conveyance of trypanosomes from insects to the vertebrate host it would seem very probable that in the case we are considering direct or mechanical transference of the trypanosomes takes place. If this is so—and future experiments will doubtless settle the point—it must be supposed that an important

[1] The curves in Charts IV, V and VIII have been coustructed by taking the mean temperature and the mean humidity for the year and expressing the respective deviations in each month as percentages in terms of the mean. In Chart VII the "mean line" corresponds to a hypothetical optimum temperature of 79° F., the monthly deviations being grouped in relation to the "mean line" as perceutages in terms of the optimum temperature.

[2] The *rattus* curve has been chosen in preference to the *decumanus* curve as being based on larger figures aud therefore probably more accurate.

factor in the successful conveyance of infection is the length of time the trypanosomes persist in an infective condition in the region of the contaminated mouth-parts of the transmitting insect. Some experiments bearing upon this question may be quoted here which illustrate the effect of drying, on the infectivity of blood containing trypanosomes. Bruce (1897) in his classical Nagana work, tested the point in an ingenious way by taking threads which had been dipped in blood containing the haematozoa; these were threaded at varying periods of time by a needle under the skin of healthy dogs. Bruce reported that

Chart IV. Showing relation of prevalence of trypanosomes to temperature.
——— = Trypanosomes in *M. rattus*.
- - - - - = Temperature.
Mean temperature = 79° F.

these experiments "would go to show that the blood of animals affected by fly disease retains its capability of transmitting the disease in a dried condition for 24 hours, but that this is exceptional and that at the end of 48 hours the blood is inert." Bruce further states that he has seen living trypanosomes and red blood corpuscles in the proboscis up to 46 hours after feeding. Kanthack, Durham and Blandford (1899) found

that complete drying of blood containing *T. brucei* rendered the blood non-infective. Examination of Chart V from this point of view makes it clear that speaking generally the period of least prevalence of trypanosome infections in rats corresponds to the season of the year during which the humidity is below the mean for the year, viz. 75 (saturation = 100) while the period of greatest prevalence of trypanosome infections corresponds to the season during which the humidity is above the mean[1].

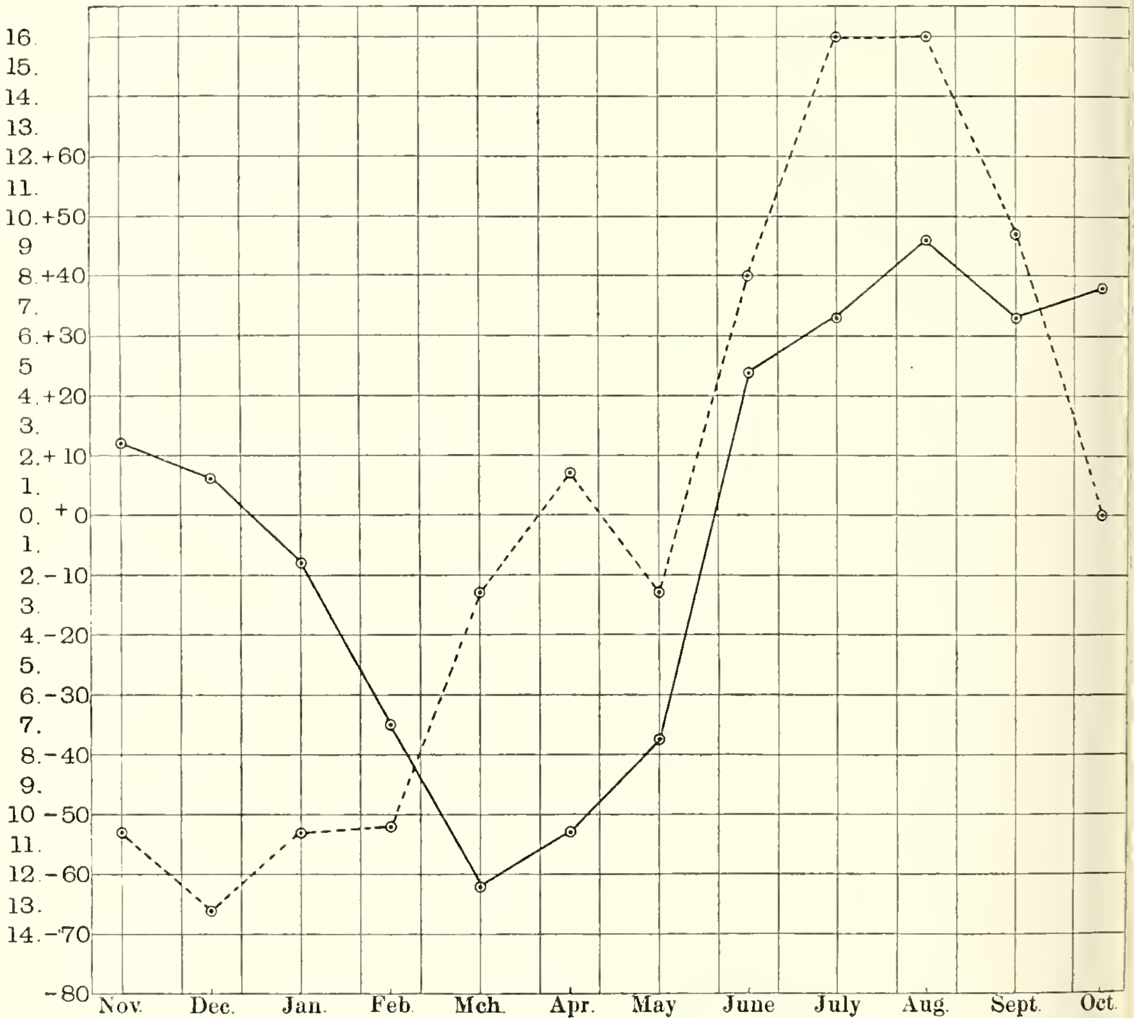

Chart V. Showing the relation of trypanosome prevalence in *Mus rattus* to atmospheric humidity.

—— = *T. lewisi* in *Mus rattus*.

- - - - - = Humidity (the mean = 75°).

[1] The rainy season in Bombay extends from June to October; the cold dry season comprising the months November to March, and the hot dry season the months April to the middle of June.

It would seem, however, that this relationship is not so striking as in the case of the temperature.

TABLE VI.

Giving mean monthly figures for temperature and humidity (saturation = 100) for Bombay and Lahore, Punjab *, 1905—1906.

	Mean temperature.		Mean humidity.	
	Bombay	Lahore	Bombay	Lahore
November 1905	80	—	67	—
December	75	57	65	89
January 1906	73	54	67	84
February	73	57	67	88
March	76	65	73	81
April	80	76	76	51
May	85	88	73	39
June	84	94	81	57
July	81	93	87	69
August	81	89	87	74
September	81	84	82	77
October	83	77	75	67
November	—	69	—	78

* The 2 Punjab villages, the rats of which were examined for the *Leucocytozoon*, are situated only a few miles from Lahore.

It has been shown above that in the case of *T. lewisi* in rats definite seasonal variations in prevalence exist. Before concluding this paper another example may be given of a "chronic" protozoal infection in rats showing similar seasonal variations. The parasite to which we refer is the *Leucocytozoon*, which was commonly observed by the Plague Research Commission (1907) in the rats (*M. rattus*) of two Punjab villages. A daily record was kept of the number of rats, in the spleen of which this parasite was found. Altogether during a year's investigation 9499 rats were searched for the parasite by the Commission and 1309 rats were found infected, a percentage for the entire year of 13·8.

We have represented graphically in Chart VI (see also Table IV) the monthly percentage figures of infected animals published in the Commission's reports. It is apparent that the prevalence curve of the *Leucocytozoon* bears a general resemblance to the curves representing the prevalence of *T. lewisi* in Bombay. It will be noted that in this

case also no definite relation appears to exist between the *Leucocytozoon* prevalence and the flea prevalence[1].

Chart VII, showing the relation of temperature to the *Leucocytozoon* prevalence, appears to us to furnish similar indications to those discussed above.

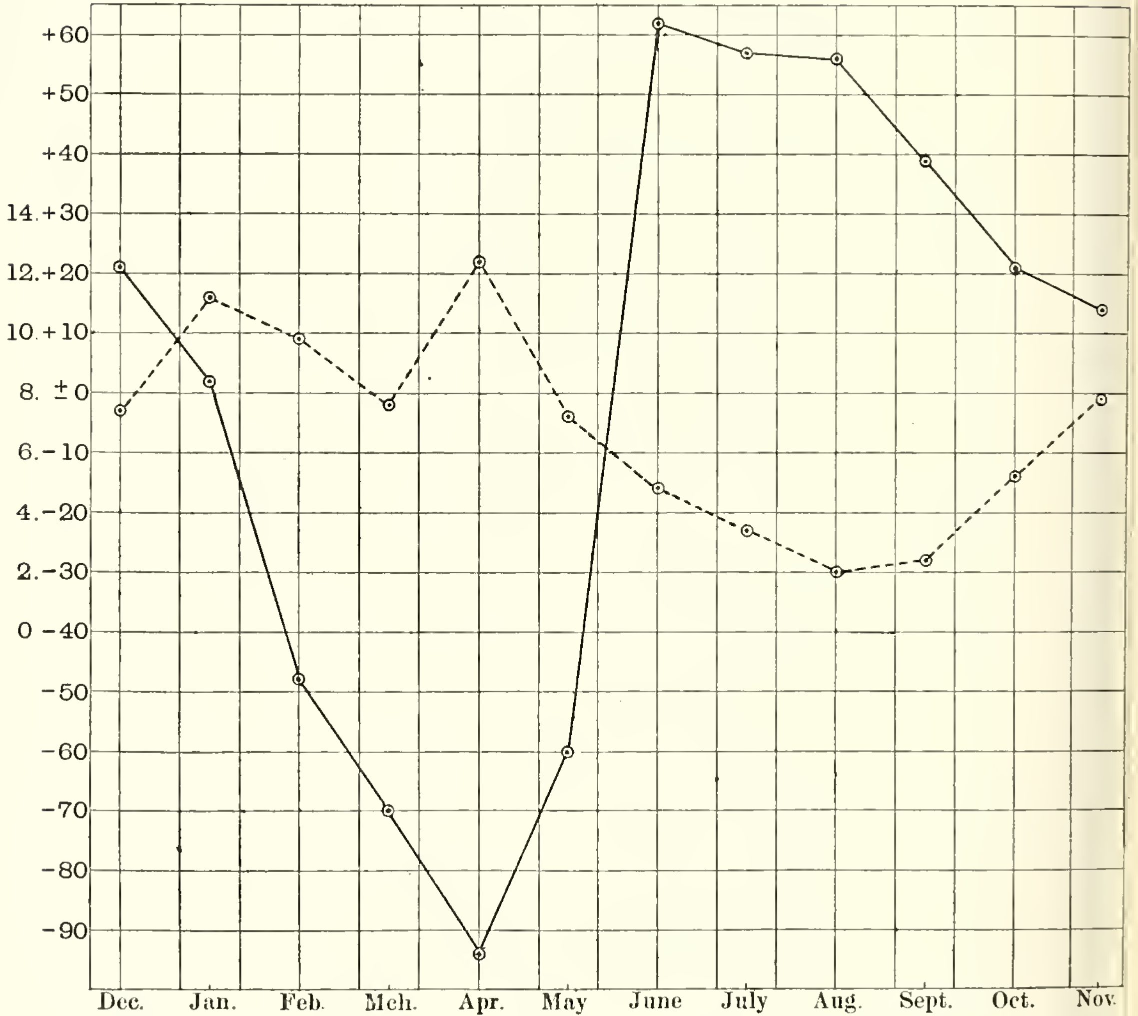

Chart VI. Prevalence of *Leucocytozoon* in *Mus rattus* in Punjab (constructed from Plague Commission's figures) and its relation to Rat flea prevalence, 1905, 1906.
——— = Leucocytozoon prevalence.
- - - - - = Rat flea prevalence (from Plague Commission's Reports).

[1] The fleas were taken from the rats examined for the *Leucocytozoon*.

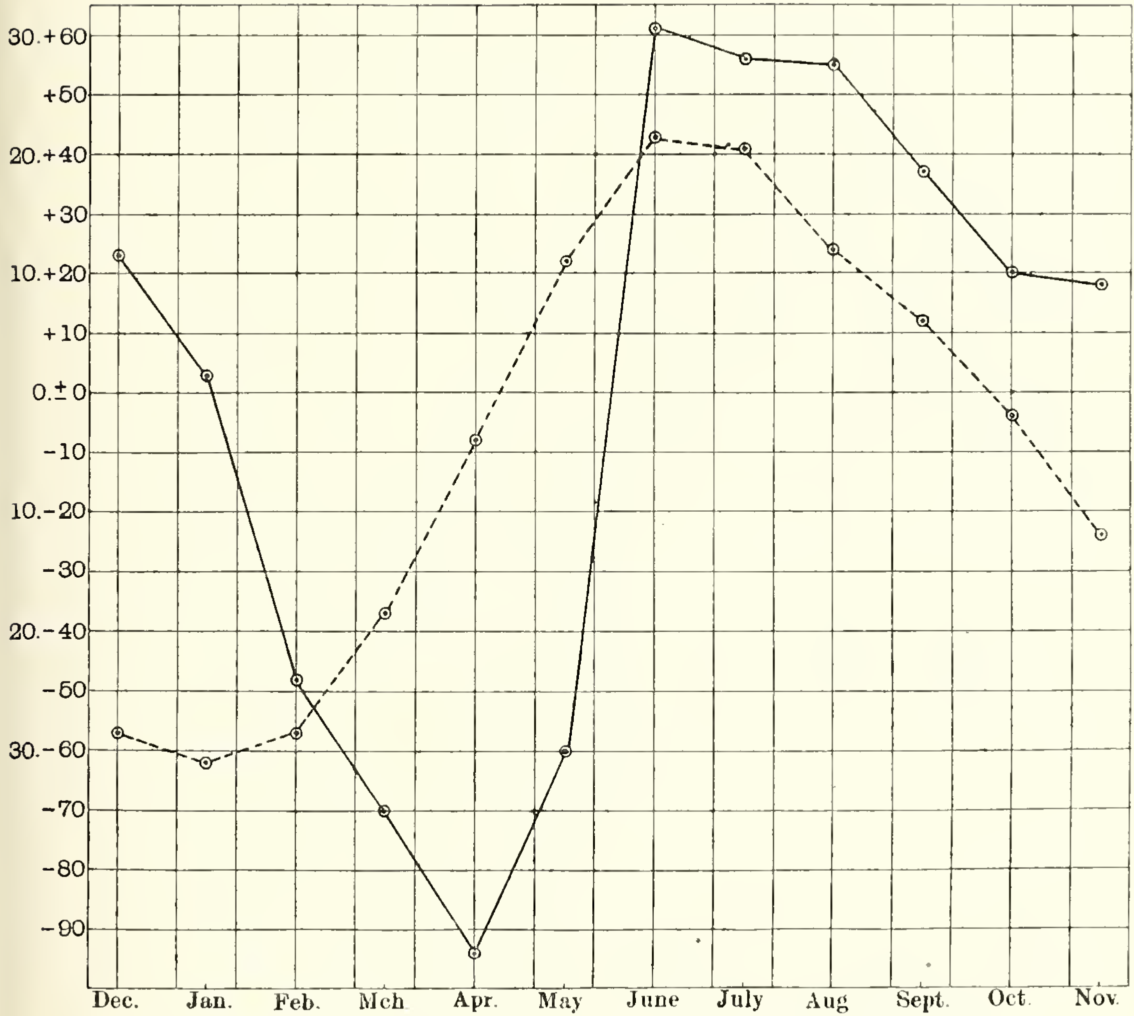

Chart VII. Showing relation of the prevalence of *Leucocytozoon* to temperature.

——= *Leucocytozoon* prevalence.

-----= Temperature.

Mean temperature = 79° F.

Chart VIII. Showing relation of *Leucocytozoon* to atmospheric humidity.
——— = *Leucocytozoon* prevalence.
- - - - - = Humidity.

Summary of Conclusions.

The above-mentioned considerations bearing upon the influence of insect prevalence and the influence of meteorological conditions on the prevalence of trypanosome infections in rats may be summarized as follows:

(1) There are definite seasonal variations in the prevalence of trypanosome infections in *M. rattus* and in *M. decumanus*.

(2) No definite correlation is forthcoming between the seasonal prevalence of *T. lewisi* and the seasonal prevalence of rat fleas. The seasonal prevalence of the transmitting insects is apparently a factor of subsidiary importance in the causation of the trypanosome prevalence.

(3) The evidence adduced above indicates that the dominant factor determining the seasonal prevalence of trypanosome infections is the atmospheric temperature, the optimum temperature approximating to 79° F. It is probable that temperature operates by influencing a developmental cycle of the trypanosomes in the transmitting insects.

(4) It is possible that in addition to temperature the atmospheric humidity may play a part by influencing the direct or mechanical transference of the infection.

It may be added in conclusion that the considerations discussed above seem to us to indicate strongly that transmission experiments with *T. lewisi* (and probably other trypanosomes) will give the best chance of success if carried out during the season of greatest prevalence of the natural infection, and further that in experimental investigations of the development of trypanosomes—pathogenic or non-pathogenic—in insects transmitting the infection to man or animals, the temperature at which the experiments are carried out should be looked upon as of the greatest importance.

Moreover, since it has been shown above that the seasonal prevalence of another parasite of the rat (a *Leucocytozoon*) bears a similar relation to temperature it is probable that the statement just made is a generalisation which will be found applicable to many protozoal infections transmitted by the agency of insects.

We have pleasure in recording our indebtedness to Lt.-Col. W. B. Bannerman, M.D., I.M.S., Director of the Bombay Plague Research Laboratory at the time these observations were made, and to Dr V. L. Manker, Mr P. S. Ramachandrier, Mr Mallet and Mr Sabnis for assistance rendered to us in various ways.

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